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EXTRATERRESTRIAL LIFE AND ITS DETECTION METHODS

Edited by A. A. Imshenetskiy

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EXTRATERRESTRIAL LIFE AND ITS DETECTION METHODS

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ANNOTATION

This collection is devoted to problems of space evolution, as well as the possibilities of "export" and "import" of life in space.

It presents a critical evaluation of several of the methods which exist at the present time for detecting life outside the Earth and the possibility of survival of lower organisms in experimental conditions simulating those in outer space.

Academician A. A. Imshenetskiy

FOREWORD

Space research has led to the development of a new discipline -- space biology. It involves the study of the effect of extreme factors that exist in space on plants and animals, examination of the possibility of transport of living matter in space with meteorites or space dust, an analysis of the possible nature of extraterrestrial life and the searching for the latter on the planets of the Solar System and finally the development of methods of sterilizing spacecraft. It is natural that space biology has made primary use of the data collected earlier by other sciences and having direct application to its own problems. The rich experience of ecologists and biophysicists makes it possible to make a correct approach to the problem of the resistance of terrestrial materials to the action of extreme factors and the prediction of the nature of extraterrestrial life. Meteoritics has provided considerable material on the chemical nature of carbonaceous chondrites, while planetology has provided valuable data on the conditions under which life could develop on the planets.

Hence, the isolation of space biology as a science has required the application of considerable data provided by various sciences. At the same time, however, theoretically new problems that did not exist previously have arisen. These include, for example, the effect of high vacuum on terrestrial materials, the study of the nature of extraterrestrial life, methods of detecting the latter, and so forth.

This collection contains both theoretical and experimental articles on various problems of space biology. The authors of these articles did not set themselves the task of providing literature surveys on this topic. Surveys of this kind will be published later in other issues.

The principal purpose of this collection is to acquaint the reader with some of the experimental studies which are going on in the field of space biology as well as with those theoretical concepts which are held by the scientists who work in this area. Undoubtedly, the landing of automatic

stations on planets in the future will provide much new information about the conditions existing on the planets and consequently corrections will have to be made in many of the ideas that are held at the present time.

A. Imshenetskiy

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EXTRATERRESTRIAL LIFE

LIFE CONDITIONS IN THE UNIVERSE

V. G. Fesenkov

ABSTRACT: On the basis of comparisons of various materials, it is shown that the chemical elements included in the composition of our galaxy have always been present in the universe and that the existence probability of some antimatter is extremely remote. The simplest hydrocarbon compounds (characteristic also of comet cores) come into existence in the interstellar medium. This is aided by dust particles consisting mostly of graphite. A complex organic substance, including some fundamental DNA-constituent groups, could have formed in carbonaceous chondrites (i.e., the oldest meteorites) in preplanetary times. It is felt that all this tends to indicate that hydrocarbons form the only foundation for life development in the universe. With the passage of time, the number of dark dwarfs continues to grow, and the universe becomes less suitable for life.

What comprises the Universe visible to us, which according to various data, /7* has been in existence for 10-15 billion years? What sort of conditions exist in it for life?

Generally speaking, the material in the Universe is concentrated in stellar systems -- galaxies, separated by distances which are reckoned in millions of light years on the average. The most massive elliptical galaxies, whose mass is gradually decreasing as the degree of their flattening increases, consist only of stars without a significant gas component. In addition, the spiral galaxies of different types consist primarily of a nucleus surrounded by more or less discrete spiral arms in which there are small percentages of gas and dust as well as stars. Finally, there are non-spiral, comparatively small galaxies with a maximum gas content.

The galaxies are grouped into individual systems which differ in composition. For example, the system with irregular outlines that is in the constellation Virgo contains galaxies which are predominantly of the spiral type,

*Numbers in the margin indicate pagination in the foreign text.

in contrast to the system in the constellation Coma Berenices. Our system also consists primarily of spiral galaxies; in fact, our own galaxy is a type Sb. The closest galaxy to us, located in the constellation Andromeda (at a distance of approximately 2 million light years), is of the same type, as are a number of other galaxies. The manner in which the galaxies were formed is a still unanswered question, but their development apparently took place many eons ago. This is indicated by the existence of surprising galactic chains, clearly unstable formations.

As established by Hubble in 1929, the universe is constantly expanding. This is indicated by the so-called red shift, i.e., the Doppler shift of the spectral lines toward the red end of the spectrum, the rate of shift being /8
proportional to the distance. The most remote objects, discovered in very recent years, are the so-called quasars, whose Doppler shift is so great that the hydrogen lines of the Lyman series, which are usually imperceptible because of the selective absorption in the terrestrial atmosphere, appear shifted to the visible portion of the spectrum. The light from the most remote quasars has been travelling toward us for more than 5 billion years; this means that it left them before our Solar System was formed.

Hence, they are located nearly at the limit of the visible Universe. Nevertheless, the spectrum of quasars contains characteristic lines of ordinary chemical elements in a different degree of ionization -- hydrogen, magnesium, oxygen, neon, etc. This indicates that the same chemical elements have always existed in the Universe, although in different amounts.

Another conclusion of theoretical significance which may be drawn on the basis of studies of the Universe consists in the fact that all of its material is in a state of constant interaction, which is especially evident in our galaxy. Observations show that powerful streams of gas are constantly being ejected from its central nucleus at velocities of 50-100 km/sec. out into the spiral arms. The stars, which represent the principal concentration of matter in the galaxy, are also ejecting gaseous material into space and generating cosmic rays, which consist primarily of protons and electrons with the maximum content of nucleii of heavier elements, which, on

entering outer space, gradually accelerate under the influence of galactic magnetic fields almost to the speed of light.

Apparently a significant number of cosmic rays also arrive from outside, especially from remote radio galaxies, but primarily from supernovae, which appear approximately every 100 years. In such an explosion, when the bursting star sometimes becomes clearly visible in the daytime sky (as was the case, for example, with the supernova Tycho Brahe (1572) in the constellation Cassiopea, energy is liberated equivalent to hundreds of billions of stars. Supernovae represent the final stage of evolution of very massive stars when the consumption of the hydrogen which serves as the basic fuel for liberation of nuclear energy undergoes catastrophic compression and the temperature at the center rises to billions of degrees. Under such conditions, there is also synthesis of various elements including the very heaviest ones, and during the explosion with ejection of an enormous mass of substance of the star these elements penetrate the surrounding cosmic space.

Thanks to processes of this kind, which have been taking place all during the existence of our galaxy, the interstellar medium has gradually been enriched with a great many heavy elements which then go to make up the substance /9
of newly forming stars. At the same time, however, the cosmic rays generated by the stars and held within the limits of the galaxy by its magnetic field irradiate all of the substance which they encounter on their path very effectively. For example, on penetrating the hardest nuclei of atoms of iron and other elements, they are enriched by nuclei of lighter elements, such as lithium, beryllium, boron; hence, they contain the latter in much greater proportions than do the stars or planets themselves.

As we know, on the basis of studies of meteorites that were performed immediately after they landed, such meteorites contain dozens of isotopes including short-lived ones that are created by the decay of various elements as well as iron under the influence of irradiation by cosmic rays that penetrate to a depth of 40-50 cm. As an example, we could mention the Arus meteorite, which was collected immediately after landing on the border between Iran and the USSR in the autumn of 1959. This meteorite was found to contain approximately 40 different isotopes, including short-lived ones that were formed

as the consequence of irradiation by cosmic rays in the course of the several million years it spent travelling in the Solar System.

A study of the meteorite substance revealed that the intensity of cosmic rays in our galaxy has remained essentially constant for the last hundred million years. Despite their being consumed in processes of collision, they are constantly being replaced by ejection from various bodies in the course of acceleration in galactic magnetic fields.

The existence of such processes clearly indicates that in the area of the Universe accessible to us, the existence of any kind of antimatter is completely impossible. It is true that these same cosmic rays contain individual positrons together with many electrons, but they may arise temporarily in processes accompanying gamma radiation. Hence, it appears strange that several authors (see for example Alfven, 1965; Vlasov, 1966) suggest that many of the stars in our galaxy may consist of antimatter. Alfven states that the galaxy closest to us -- the Andromeda Nebula -- also consists of antimatter. However, all the data which we have on the continuous interaction of substances within our galaxy and beyond its limits indicate the absolute impossibility of this. Hoyle (1968) considers it to be a sufficient argument against the existence of antimatter that the fission of particles and antiparticles following their creation in nuclear processes cannot take place. Especially strange are the opinions expressed very recently (La Paz, 1968) that individual comets may consist of antimatter and that in particular the explosion which took place when the so-called Tungus meteorite landed on 30 June 1908 was also caused by antimatter. /10

Another important question which is faced by investigators in the field of exobiology is whether or not life on a basis other than carbon could develop and exist somewhere in the Universe. At the present time, no one can answer whether or not life is possible in our Solar System in this respect.

Considerable success in determining the structure and composition of the interstellar medium was achieved by the use of radio methods. On the 21 cm wavelength, it has been possible to confirm the existence of neutral hydrogen in the spiral arms of the galaxy and its approximate content was determined.

In addition, ionized hydrogen has also been found to form extensive areas that develop in it under the influence of irradiation of interstellar medium by bright, comparatively young stars. Areas of hydroxyl (OH) have been discovered which were first found in the absorption line at a wavelength of approximately 18 cm, and then in the emission as well. Emission lines of other elements at very high levels of excitation have been found in the radio band.

Within the limits of our galaxy, interstellar gas appears in the optical region of the spectrum in such a manner that it causes stationary lines of various elements (for example, ionized calcium). In addition, absorption bands have been found in interstellar space which no one has yet been able to relate to any molecular compounds; in all probability, they characterize the composition of particles of interstellar dust. An insignificant amount of this interstellar dust absorbs light strongly and is able to conceal completely the central nucleus of our galaxy in optical rays; this nucleus is located in the direction of the constellation Sagittarius. In addition, the interstellar fragments of dust, which are cooled to a very low temperature (3-5°K), are capable of causing considerable cooling of the interstellar gas, leading to its condensation.

According to the detailed studies performed by a number of authors (Nandy, 1967; Hoyle, 1968, etc.), we can assume that interstellar dust of this kind is constantly being enriched with grains of graphite, which are formed in the atmospheres of red giants (type N) and expelled into outer space by the pressure of light. In some areas, these graphite particles are covered with a thin coating of ice (for example, in the absorbent clouds in the constellation Perseus) and always contain admixtures of other precipitated elements.

Another reason for the abrupt cooling of the gas in the interstellar medium, leading to its condensation, is the admixture of small amounts of oxygen, nitrogen and neon, which are excited by the absorption of thermal energy in collisions with atoms of other elements, reaching high levels, and then emitting forbidden lines in the optical and radio frequency ranges. Although these elements are present in only a very slight amount, they are able /11
to reduce the temperature of even very hot regions of ionized hydrogen by an entire order of magnitude, approximately to 10,000° K.

The interstellar medium is far from uniform. It contains turbulent processes and local heating under the influence of ionization of gas. Flares from supernovae create shock waves which propagate at supersonic velocities, so that the density of the gas in such a wave is increased several orders of magnitude and this may be of great significance for the formation of gravitationally bonded condensations. A convenient example of a process of this kind is the filaments in the constellation Cygnus, which are spreading at velocities of approximately 100 km/sec from a single center at which a supernova exploded 70,000 years ago. Among these filaments, we can find a number of stellar chains oriented in the same fashion and representing unstable formations that developed recently. Many similar filaments have been photographed in various portions of the sky by Shayn and Gaze (1952).

If a cloud of gas and dust with high density is located in the path of the shock wave which characterizes the propagation of an ionized front, the former remains unionized, is compressed under the pressure of the hot gas and is converted to a dense dark formation -- a globule which is the nucleus of a star, and which in a very short time may be compressed as a result of gravitational collapse and be converted into a selfluminescent body. A great many such globules, usually associated with nebulae, were discovered in the United States by Bok and studied by Rozhkovskiy (1962).

Condensations have developed rapidly in which significant changes have taken place in only a few years; these are the so-called Herbig-Garo objects, which are found in particular in the vicinity of the Orion Nebula, where intense stellar formation is going on at the present time.

Thus, for example, Beklin and Neugebauer recently (1968) discovered infrared radiation in this nebula, coming from some object that was apparently in a stage of pregravitational collapse. Near the recently developed type T stars, which are particularly numerous in this nebula, Mendoza in 1962 detected the presence of considerable infrared radiation, which must come from a compressed dust cloud with a temperature of about 700°K, apparently consisting of material for the formation of planets. It was recently shown (Menon, 1967) that hydroxyl (OH) radiation only develops near forming stars and comes from objects

of negligibly small angular size (Shklovskiy, 1966, 1967). We must take into account the fact that at the boundary of the area of ionized hydrogen, surrounding a star which is just developing, there is a decay of molecules of H_2O and the formation of hydroxyl OH. This is similar to the development of hydroxyl in the upper atmosphere from water vapor under the influence of high-frequency solar radiation. /12

As we mentioned earlier, the Orion Nebula is especially characterized by the fact that star formation is going on in it at the present time. For example, in 1936 the star FV Orionis appeared there and similar phenomena may occur in this nebula every 500-1,000 years (Herbig, 1967). What is the structure of such a nebula like? A large quantity of fine absorbent material is associated with it. Detailed spectroscopic studies have revealed that it consists of a number of inhomogeneities which occupy no more than 1% of the total volume (Osterbrock, Fleischer). According to the estimates made by Idlis, inhomogeneities of this kind are equivalent in mass to comets, i.e., they have masses on the order of 10^{26}-10^{28} grams.

Would it be correct to assume that such nebulae actually consist of comets? This would not be absolutely correct, since comets which make up a portion of the extended circumsolar cloud measuring up to 1,500 radii of the Earth's orbit, although they may remain fixed in the conditions of interstellar medium for a practically unlimited length of time, contain very insignificant amounts of hydrogen and this mainly in the form of various compounds. Spectrometric data indicate that comet nuclei are made up of CN, CH, CH_3, C_2, C_3, OH, CO, NH, CH^+, CO^+ and Na, but the so-called "parent compounds" which make up their inner portions must be more complex, for example: HCN, NH_2, H_2O, CO, C_2N_2, CH_4, C_2H_2 and other hydrocarbons with admixtures of various heavy elements -- Fe, Ni, Mg, Ca.

Accumulations with compositions of this kind obviously may develop anywhere in space and serve as primary material for the formation of stars and planets. The unavoidable mutual collisions between such condensations, forming along irregular orbits around a common center of gravitation, lead to the rapid separation of the central condensation, which is then changed into a star when there is sufficient mass.

It should be pointed out, however, that we still do not have a completely clear idea regarding the mechanism of formation of the comet which makes up a portion of the extended circumsolar cloud, although it is clear to everyone that unstable bodies of small size like these could not develop within the Solar System. Vsekhsvyatskiy, however, proposes the rather novel idea that comets were ejected and continued to be ejected from the cores of planets and their satellites during some kind of internal cataclysms (Vsekhsvyatskiy, 1967). His main argument is the fact that many comets appear to have developed abruptly at slight distances from Jupiter and then describe more or less elongated orbits. However, all such cases were studied in detail by Kazimirchak-Polonskaya (1968), who used computers to calculate the evolution of cometary /13
orbits under the influence of perturbations caused by all the planets in the Solar System from Venus to Pluto. She showed that this may be accompanied by a significant change in the shape and size of the orbit, that the comet may shift from one planetary family to another, and that a comet may abruptly enter the field of visibility and become accessible to observation or, on the other hand, may disappear from the observer's view. Kazimirchak-Polonskaya showed that for hundreds of years prior to its discovery, the Lexell comet moved along a slightly eccentric orbit with a period of 10 years having a very high perihelic distance equal to three astronomical units. In 1767, this comet came within only 0.02 astronomical units of Jupiter and as a result of its severe perturbations began to describe an elongated ellipse around the Sun with a period of 5.6 years. In 1770, this comet came within 0.015 astronomical units of the Earth and was then discovered.

Nine years later, the Lexell comet again penetrated deeply into the sphere of influence of Jupiter, coming within a negligibly small distance (0.0015 astronomical units) of this gigantic planet. It was then near its aphelion and was still moving away from the Sun. The attraction of Jupiter considerably increased its velocity and actually expelled this comet beyond the limits of the Solar System.

The role of comets in the past was more important, when the population of the circumsolar cometary cloud was much greater. Even with the current excess

of comets, we can expect that in the course of its history the Earth has collided with comets no less than 100 times and collected various organic compounds from them on these occasions (Oró, 1965).

In reality, the number of encounters of comets with the Earth and other planets was much greater, if we take into account the incomparably high level of richness which the circumsolar cometary cloud enjoyed in the past.

We could point out the following data in connection with the role of comets, the ancestors of the most ancient meteoritic substance -- carbonaceous chondrites -- in the origin of life in the Universe.

As indicated by numerous experiments performed by Studler, Hayashi, Anders et al., (1968), dealing with the formation of complex organic compounds found in carbonaceous chondrites, similar compounds are found in equivalent reactions of the Fischer-Tropsch type from the simplest hydrocarbons and nitrogen compounds with the necessary presence of catalysts, for example of even a negligible amount of meteoritic iron, and under conditions of local brief heating to temperatures of about 800-900°K with subsequent rapid cooling in the course of several hours or at least days. With longer heating, the complex compounds which are formed will break up.

We can imagine that the reason for this overheating might be flares from /14
a still incompletely formed sun. According to the calculations performed in 1968 by Hayashi, a star with the mass of a sun, immediately after its expulsion from the interstellar gas, and reaching the stage of gravitational collapse, is initially nontransparent, i.e., it turns into a globule, and initially radiates only a small fraction of its current radiation. Later, a shock wave develops within its mass and, reaching the surface, produces a large flare, suddenly increasing the radiation flux by thousands of times. This is followed by a sharp collapse as the star gradually enters a curve on the main sequence and becomes stable. This result is not given by other calculation models, and the star of solar mass develops a stable condition with comparatively slight increase in radiation flux (Herbig, 1967). At the same time, we cannot deny the possibility that our Sun, soon after its formation, was changed by numerous brief explosions.

Another reason for the brief overheating mentioned above, which appears more probable, lies in the unavoidable collisions between the numerous cometary nuclei. As the result of braking, these comparatively small bodies were overheated and expanded with subsequent rapid cooling. This meant that in the primary circumsolar nebula there arose complex organic compounds -- the bases of DNA -- and the conditions were created for the origin of life. This process was obviously general throughout the Universe. It is scarcely reasonable to expect that life developed in parallel with this and on some other base, for example on the basis of silicon compounds or other elements. This is not supported by the experiments, by the study of the material making up meteorites or even by our general concepts regarding the phenomena taking place in the Universe.

Thus, all of the available data indicates that life is developing and changing everywhere in the Universe on the basis of carbon alone.

Earlier, we discussed the processes of interaction between the various component parts of the galaxy. However, there is incomplete periodicity in these processes. The Universe is continuously expanding, the distances between galaxies are increasing and the density of the intergalactic material and its temperature are gradually decreasing. In addition, although the spiral arms are continuing to remain "frozen" in their magnetic fields and participate in the general rotation around the galactic center, the mass of the central nucleus apparently is decreasing constantly due to the continuous ejection of material. Star formation is taking place in the spiral arms, but if the massive stars exist for a comparatively short time and eject a considerable /15
portion of their mass into interstellar space after using up their supply of fuel which serves to maintain nuclear reactions, the interstellar space will be enriched with heavy elements and a star of small mass will remain in a stable condition, gradually changing to a white or even dark dwarf.

As was pointed out earlier by Chandrasekar, the specific mass below which the star remains in a stable condition and is not subject to catastrophic explosions amounts to 1.1 solar masses. The smaller the mass of the star and consequently the lower its temperature and internal pressure, the less able it is to develop nuclear energy and the slower its evolution will take place along the curve of the main sequence. The lower level of stellar mass, depending on

its chemical composition, amounts to 0.07-0.1 Solar masses. With a mass less than this limit, a star will not follow the normal path of stellar evolution and will simply become a black dwarf or small planet, in which a certain heating of the substance will be performed by the decay of ordinary radioactive elements. The structure and evolution of stars of very small mass was studied later by Grossman, Grabozhka and others. Kumor feels that the invisible satellites of 61 Cygni, 2354, Barnard's star and others are in a dwarf state of this kind and are essentially large planets. Deych (1967) reached a similar conclusion somewhat earlier on the basis of his observations conducted at the Pulkovo Observatory.

In general, a large number of such bodies may be said to have accumulated in our galaxy during the 10-15 billion years of its existence. They are nearly inaccessible to observation and we know of their existence primarily through their total gravitational effect. Their total number, according to several estimates, makes up about 40% of the mass of our entire galaxy. In the immediate vicinity of the Sun, extending to distances of 5 parsecs (1 parsec is equal to 206,265 astronomical units), the overwhelming majority of stars have very small masses and consequently are of negligible brightness. The number of black dwarfs that have been found is probably greater.

Hence, as the Universe ages, conditions for existence of life in it become less suitable. It is difficult to say what will happen in the future. It may be that the continuous expansion of the Universe will be replaced by contraction, a violet shift, and all the material contained in it will gradually become heated, until it reaches the state of a hot gas. This problem is closely linked to the selection of a cosmological system and has not yet been solved.

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GENESIS OF LIFE ON AND BEYOND THE EARTH

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ABSTRACT: The possibility of extraterrestrial life is considered in the light of the materialistic view of life as one of the stages in the general process of matter development. The merits of carbon as the chemical foundation of life are discussed. Arguments are presented in favor of life existence only on a single type of cosmic body, i.e., on planets alone, the presence of water being supposed to form the main prerequisite. Confidence is expressed in the capability of spaceflight to help elucidate many of the moot questions.

The problem of life in the Universe has interested the human mind for many centuries.

Very often, our ideas concerning the possibility of life beyond the Earth, and the forms of it which we may encounter in outer space or on other heavenly bodies are based only a study of data on physical and chemical conditions existing on these bodies at the present time. However, the inadequacy of such a method for solving the problem of life in the Universe is obvious. Concrete forms of life are the result of those external conditions in which they develop and grow. The external medium forms the organism, constituting extremely complete and frequently very complex systems of accommodations. If /17 there were no life on our planet outside the ocean (land plants and animals), it would be very difficult theoretically to imagine the possibility of such life.

The solution of the problem of life in the Universe not only consists of determining whether or not the type of living forms which we know could live on a given heavenly body under the conditions existing on it at the present time, but rather of determining whether or not in the process of its evolution there was a possibility of the development and growth of the type of complex form of motion of matter which we call life. Every heavenly body, especially any planet, develops in the course of its existence and the conditions which are characteristic of it at the present time are by no means permanent. At a

certain stage of development of a planet, it may be subjected to conditions which are favorable for the development of life, which then comes into existence and gradually assists in these changes.

Hence, our ideas regarding the possibility of life beyond the Earth and its concrete forms must be irrevocably linked to the study of the entire process of development of matter. At the present time, it has become absolutely clear that the development of life on Earth was no "happy accident". The development of life is an inherent part of the entire orderly process of development of the Universe, in which each subsequent stage is inherently linked to the preceding and may be understood only in the light of a study of that preceding stage.

In trying to get an idea of the general picture of the development of the Universe from the evolution of the elements and prior to the development of thinking matter, we must free ourselves of the idea that this evolution followed some single straight line. It has followed and is following at the present time different paths on different celestial objects. Therefore, we can think of it schematically as a system of parallel or divergent paths, whose individual branchings may lead to very complex and complete forms of movement of matter. We still do not know anything about many of these forms, and in many cases are not even aware of their existence. But it is impossible to accept as correct the currently rather widespread tendency to immediately view all of these forms as life even in the case when there is a basis for assuming that the development and growth of this form took place along theoretically different pathways than those followed by life in the course of its development here on Earth.

Thus, for example, we frequently read in the popular literature articles about the possibility of the existence of life, whose basis is not carbon but silicon. This idea is based only on the fact that the two elements are located /18
close together in the periodic table of the elements. However, as was demonstrated on the basis of studies of molecular orbits by S. and A. Pullman (1964), silicon can neither adopt an intramolecular structure which is characteristic of organic biopolymers nor can those reactions take place which are necessary for the development of biological metabolism.

As a matter of fact, under terrestrial conditions silicon compounds cannot be compared with the organic substances which gave rise to life. Finally, we can assume that under different conditions which are completely unknown to us, the evolution of silicon compounds took place under completely different conditions than on Earth and led to the development of very complete forms of movement of matter. However, if this really did take place somewhere, why is it absolutely necessary to regard these forms as life?

Life is the result of only one of the above mentioned numerous branches of material development. It is characterized by its own specific pathways of growth and development, pathways which are linked to the evolution of carbon compounds.

We can assume a priori that the original substances for this evolution, especially the hydrocarbons and their near derivatives which form the basis of all organic life, must be very widely distributed through the Universe. On the one hand, this is dictated by the exclusive quantitative predominance of hydrogen in the cosmos and on the other hand the fact that no specific conditions (for example, explosions of supernovae) are required for the development of carbon (in contrast to heavy elements). It took place in the stable process of stellar radiation (Merill, 1959). Hence, hydrogen may actually be detected when studying the spectra of all classes of stars, especially the oldest branches of the stellar population and our galaxy. In stellar atmospheres which have the highest temperatures, conditions do exist for the formation of chemical compounds, but hydrocarbon bands appear with ever increasing clarity in the spectra of type A stars and in all subsequent types. In particular, the atmosphere of our Sun contains compounds of carbon with hydrogen, as well as with nitrogen (cyan) (von Klueber, 1931).

Hence, hydrogen compounds are very widely spread over the surfaces of stars with temperatures of several thousands of degrees and very high gravitation. However, we can also find hydrocarbons in other diverse objects in space, under much different conditions of existence, for example in clouds of interstellar gas and dust material, with extremely low gravitation and temperatures close to absolute zero (Bates and Spitzer, 1951).

Consequently, this original carbon material, which was necessary for the development both of our terrestrial life and for its analogs on other heavenly bodies, is found everywhere; its absence cannot serve as an obstacle to the formation of life, and the entire question boils down to how this material evolved further on a specific object in space. /19

On the surface of our planet, the original hydrocarbons and cyanides, as well as their close oxygen, nitrogen, sulphur and phosphorus derivatives, using external sources of energy (for example, ultraviolet light, electrical discharges, local heating) gradually changed into more and more complex organic substances, first into such monomers as (for example) amino acids, nitrogen bases, sugar, and then their polymers, such as proteins and nucleic acids. The combination of these polymers into multimolecular systems and subsequent evolution of these systems, based on their prebiological natural selection, took place in the same fashion as the formation of probionts and eobionts took place; the latter are the ancestors of all life on Earth (Oparin, 1966).

It is understood that this kind of sequence of evolution of carbon compounds may take place only under the conditions of a comparatively narrow framework of external conditions: temperature, gravitation, light, etc. In particular, free water in the ambient environment is of particular importance. The majority of laboratory experiments which simulate the individual stages of the evolutionary process of organic substances and the multimolecular systems which develop in them that we have mentioned above have involved the necessary participation of water (Pavlovskaya, Pasynskiy, 1964). It is true that Fox et al. (Harada, Fox, 1964) synthesized amino acids from primitive gases in laboratory experiments simulating volcanic conditions. Fox et al. (1959) succeeded, by heating a mixture of amino acids to 150-180°, in synthesizing their high-molecular polymers, the so-called proteinoids, in the absence of water. However, according to Fox, further evolution of these substances involves the formation of microspheres and must necessarily take place under conditions of an aqueous medium.

It is clear that the nature of the development of cosmic objects from stellar and interstellar gas and dust material differs essentially from all

of that which is required for the development of life on Earth. Hence, we must limit our attempts at looking for suggested extraterrestrial life to the limits of the planetary systems.

However, these systems cannot be formed without the participation of heavy elements which developed much later than the first accumulations of stellar population of our galaxy. Bodies of sufficiently great mass, like /20
stars, may be formed exclusively from light elements, even almost completely from pure hydrogen. In all probability, this was also the case at the very beginning of existence of the galactic system. Such a mass is completely stable and with sufficient density in it, very simple thermonuclear reactions involving the change of hydrogen to helium take place , serving as the source of stellar energy. But it is completely impossible for there to be a body with the size of our Earth that is completely formed of pure hydrogen, since the mass of these bodies is insufficient to keep the hydrogen from dissipating into space. From this we can conclude that during the first billions of years of existence of our galaxy, when heavy elements had not yet been formed in sufficient amounts in it, planetary systems analogous to those existing at the present time could not have existed and consequently there could not have been life which developed in the course of the evolutionary process of these planets.

Hence, we must conclude that the development of life in the Universe is limited only to the limits of planetary systems of stars of rather late types, already possessing all of the elements in the periodic table.

However, even within the limits of planetary systems, by no means all of their members could develop under conditions that would ensure the possibility of development of life upon them. This has to do both with the chemical nature of the substance from which a particular planet or asteroid was formed as well as with their dimensions, distance from the central luminary, level of radio-activity, and so forth.

According to the calculations of Brown (1964), thousands of visible stars may have planetary systems, with an average of two planets in a zone where the conditions of temperature and illumination are suitable for the development of life. If their Sun, as pointed out by Bernal (1967), is larger or hotter

than ours, such planets would be located at a greater distance from it, and at least one of them will necessarily have to satisfy all the required conditions. We can state with less certainty that the dimensions of such planets will be suitable for the development of a free hydrosphere, but the probability of existence of this condition is very significant. Further, Bernal comes to the conclusion that the initial development of life may have taken place in our galaxy at least 10 billion times.

Even if we consider this number to be too high and reduce it by several orders of magnitude, it becomes theoretically necessary to recognize the high probability of a very broad distribution of life throughout the Universe, beyond the limits of our planet. However, regardless of such a fact, as of the present time we have still been unsuccessful in detecting any reliable signs of life outside the Earth (not only living organisms, but their remains or signs of life) both on the scale of the Universe or within the limits of /21
our planetary system. However, within the framework of the latter, we do have some actual material which suggests that on some extraterrestrial objects, as here on Earth, evolution of carbon compounds has taken place, although we still do not know how far it may have advanced in individual cases.

It is usually assumed that both the Earth and the other planets in our system were formed from a material that composed a discoidal cloud of gas and dust, which at one time surrounded our luminary. According to Brown (1950), all of the substances that made up this cloud can be divided into the following three groups. The so-called gas group includes hydrogen, helium and the noble gases, which retain their gaseous state at temperatures close to absolute zero. Then we must create the "ice" group, including those substances which were formed from carbon, nitrogen, oxygen (plus hydrogen), as well as those like ammonia, methane and water. Finally, there is a group which we shall conditionally refer to as "Earth", made up of compounds of silicon, magnesium, iron and other elements.

The temperature of this protoplanetary cloud was considerably dependent on the one hand on solar radiation and on the other hand on the loss of heat by the clouds through radiation into interstellar space. Therefore, its

temperature differed considerably at the periphery and in the central areas. This fact was also indicated by the redistribution of substances in the discoidal clouds during the formation of planets in it (Whipple, 1964).

Close to the periphery, where intensive cooling was taking place, the substances of the "ice" group condensed at high speed, "froze" into "Earth" particles, forming so-called cometesimalia. In the vicinity of the orbit of Jupiter and further toward the periphery, the gas, dust and cometesimalia rapidly combined into the major planets, and partly into comets, which formed at the limit of our planetary system under conditions of a nearly interstellar medium.

A confirmation of the above is found in the excessive content of such substances as methane and ammonia in the composition of the major planets. Of course, we cannot exclude the possibility that there was a further evolution of these substances, with their transformation into more complex organic compounds. This is indicated particularly by the discovery on Jupiter of the so-called "red spot", which is a constant part of the planetary atmosphere. It is assumed that its color is caused by the presence of complex and rather high-molecular carbon compounds (Bernal, 1964).

However, the considerable difference between the conditions which exist on the major planets and those which we have on Earth makes it highly likely that evolution of organic compounds could take place in those areas of the solar /22
system along the same pathways as on our planet. In addition, it is scarcely possible to think of comets as being hospitable to life. However, the carbon compounds which are included in their composition (especially cyanogen), being found on Earth, could serve, as stated by Oro (1965), as material for the synthesis of biologically important organic substances on the surface of our planet.

The original material for the formation of planets of the Earth type took place differently than at the periphery of the discoidal cloud. In these areas comparatively close to the Sun, the gas component of the cloud was nearly completely gone, "ice" was retained only partially, and the principal material was that in the "Earth" group. The accumulation of dust particles

composed of this material led to the formation of so-called planetesimalia -- small bodies that incorporated all of the nonvolatile substances of the primary cloud, as well as a small amount of components from the "ice" group. Further combination of the planetesimalia led to the formation of the planets of the Earth types as well as the asteroids -- small cosmic bodies formed in our planetary system between the orbits of Mars and Jupiter.

According to an opinion which is widely held at the present time, the asteroids themselves or other comparatively small bits of matter (measuring 100-1,000 km in radius) served as the "ancestral bodies" for the formation of various types of meteorites the only ones available prior to the study of the lunar samples collected by "Apollo-11" (Ponnamperuma, 1970), samples of extra-terrestrial matter which we can now subject not only to chemical but also to mineralogical analysis, making it possible for us to get a better idea of the cosmic history of carbon compounds (Mueller, 1967).

A study of the various radioactive isotopes present in meteorites makes it possible to date individual events in their history. The growth of the heavy elements included in their composition is the same as that of other bodies in the Solar System. The time which has passed since the hardening of the meteorite substance is reckoned on the basis of a study of the lead, rubidium and strontium isotopes to be 4.5 billion years. The magnitude of loss of certain volatile radiogenic isotopes indicates that a portion of the substance of the meteorite material was at a comparatively high temperature for a short time. The so-called cosmic age of the meteorites, i.e., the time which passed from the moment of their formation from the ancestral bodies as a result of collision of the latter or in the process of explosive volcanism, is determined to be only tens or hundreds of millions of years. Regularities in the composition and internal structure of meteorites indicate different conditions of formation of different groups of meteorites and their formation from different zones of the original bodies that formed them, which were of asteroid size.

The carbon compounds in which we are interested are found in many meteorites, but are particularly plentiful in the carbonaceous chondrites, in which (according to Vdovykin, 1967) they make up up to 5-7% of the organic substance. /23

All carbonaceous chondrites consist of a mixture of mineral grains (chondrules), which can only be formed at high temperatures, and a principal mass which was never heated above 100-300° all through the cosmic history of these meteorites. The mechanism of mixing of such high and low temperature phases still cannot be considered explained, but the majority of authors feel that carbonaceous chondrites correspond to the substance of the upper zones of the ancestral asteroid bodies.

At the present time, the carbon substance of carbonaceous chondrites has been studied and analyzed in detail by many investigators. They have found various organic compounds, which are also known under our terrestrial conditions. These consist primarily of hydrocarbons of the C_{15}-C_{29} series, paraffins and cycloalkanes. In this connection, we should also mention the aromatic compounds of the benzene series and the multinucleate hydrocarbons -- phenanthrenes, pyrenes, chrysenes. Aromatic and fatty acids of the $C_nH_{2n}O_2$ type have been identified in many carbonaceous chondrites. Among the latter, acids with an even number of carbon atoms in the chain predominate. Carbonaceous chondrites also contain sulfurous derivatives of hydrocarbon (heterocyclic compounds of thiophenes).

As far as biologically important compounds are concerned, carbonaceous chondrites have been found to contain amino acids, especially serine, glycene, alanine, leucine, treonine, asparagenic and glutaminic acids[1]. Anadine derivatives have also been found in meteorites: melamine, ammeline, adenine and guanine, with the last two playing an important role in the synthesis of nucleic acids. Finally, together with fatty acids, carbonaceous chondrites have been found to contain carbohydrates (in particular, mannose).

Recently, Nagy et al. (1961) made an attempt to treat organic substances of carbonaceous chondrites as the result of vital activity of living substances that lived upon them or on their ancestral asteroids at one time. The authors try to support this view by pointing out that the meteorites had been found to contain structures ("organized elements") which could have been the remains

[1] However, indications have been found recently that these substances were introduced from outside during analysis of the meteorites.

of extraterrestrial life forms. However, this opinion was overturned by many investigations (Anders, Fitch, 1962), which showed that the "organized elements" of Nagy had no relationship to living structures and represented mainly mineral particles, while in other cases that were comparatively rare they were the results of accumulation of terrestrial microbes, which could have penetrated deep into the cracks of the meteorites even prior to their fall. /24

In addition, the abiogenic nature of the meteoritic organics is supported by many facts which were determined through careful analysis; certain characteristics of their chemical composition, the absence of optical activity, the isotopic composition of the carbon and hydrogen, as well as the nature of the distribution of the carbon compounds.

Hence, using the example of carbonaceous chondrites, we can say that under conditions which differ significantly from those on Earth, carbon compounds could have evolved within rather wide limits and could have been changed to those complex organic substances which on Earth have served as material for the formation of life.

However, this evolution has not led to the development of life. The reasons for this are very numerous. However, in the opinion of Mueller, the most important one was "drought" -- the absence of free water on the meteorites or on the bodies which gave rise to them. We find only water that is linked with hydrated rocks in the composition of carbonaceous chondrites.

The most important problem in determining the minimum conditions which are required for the development of life is the role of water in the cosmic history of "ancestral bodies" (Mueller, 1967). Judging by the fact that all meteorites lack typical precipitated structures and also contain grains of olivine and other readily hydrolyzed silicates, free water must have existed during the entire cosmogonic history of the "ancestral bodies" for only a very short time, no more than decades or centuries at most.

The Mueller "drought" could have a similar negative significance for the development of life on other heavenly bodies located in the vicinity of our planet, especially our natural satellite, the Moon, and to a certain degree on the planet Mars.

It is true that Bernal points out that water can be kept beneath the permafrost layer or in frozen mixtures of dust and ice, which prevent its evaporation, as is the case for the basis of certain immobile glaciers in Antarctica; he therefore suggests that it is possible for water to exist beneath many of the lunar craters.

Of course, this is only a suggestion, and it is quite likely that even if there are carbon compounds on the Moon (although none have yet been found in the analyses of the surface layer of lunar dust) (Ponnamperuma, 1970), their evolution has not proceeded further than we can detect by meteorites.

The photographs of Mars that were published in 1965, taken with the aid of the Mariner interplanetary automatic station, indicate that even on this planet, with a diameter of 6,620 km, the craters which are characteristic of /25
the Moon are predominant, and deposits of water are either completely absent or are found in extremely small amounts. However, many investigators of Mars have reached the conclusion that there are "moist areas" on it or even very small and narrow watercourses on the surface of the ice coating or beneath it. In this connection, we can suggest that evolution of carbon compounds has taken place on Mars to a much greater degree than on the asteroids or the Moon.

The fact that Sinton (1959) has observed bands which are characteristic of organic molecules in the infrared reflected light from Mars is very interesting from this standpoint; these bands are characteristic only of the dark areas of the planet and are missing on its bright sections. Colthup (1961) interprets these bands as an indication of the presence on Mars of organic aldehydes, especially acetaldehyde, which could develop here through the metabolism of anaerobic organisms, in a fashion similar to that in which they develop at a certain stage of alcoholic fermentation. However, Young et al. (1964) performed model experiments which demonstrated the possibility of abiogenic formation of organic substances and their polymers on Mars. In a chamber which was specially constructed for this purpose, with appropriate temperature, ultraviolet radiation and limonite in the form of soil, there was considerable fixation of CO_2 and the formation of acetaldehydes and various sugars (pentose and hexose).

In the opinion of these authors, organic substances synthesized abiogenically in the Martian atmosphere (including acetaldehydes) have accumulated on the surface of the planet, piled up on its dark areas, while in the bright areas they may only break up, which explains Sinton's phenomenon.

Hence, the question of how far the evolution of organic matter has proceeded on Mars and whether or not life could arise and develop there still remains an open one. We can hope that the continuing successes in the conquest of space will provide a scientifically based answer to this question (Ponnamperuma, Klein, 1970).

Very great hopes in regard to the existence of life were recently placed on Venus, which in many respects (size, mass) is very similar to the Earth and is our closest neighbor in the planetary system. However, the further the detailed study of this planet has progressed, the less bright these hopes have become. The direct measurements of the density, temperature and chemical composition of the Venusian atmosphere that were performed by the scientific laboratory of the automatic station "Venus-4" showed that the conditions on this planet are quite different from those on Earth. Near the surface of Venus, the temperature is about 280°, while the atmospheric pressure is roughly 15 times greater than on the Earth. In terms of its chemical composition, /26
the atmosphere of Venus consists almost wholly of carbon dioxide gas, and the content of oxygen and water vapor amounts to only about 1.5%, while no noticeable traces of nitrogen were found.

In the light of these data, it is very difficult to consider it possible that there is life on Venus. Apparently, evolution of carbon compounds here has followed a completely different group of pathways than on the Earth, due to the very different thermal history of these planets (Fesenkov, 1964).

Summing up all of the above, it is most evident that although we are theoretically convinced of the existence of extraterrestrial life, still we have not been able to find it anywhere (practically speaking) beyond the limits of our planet with complete certainty. However, we are now gathering considerable factual material which indicates that the initial stages of evolution of carbon compounds which give rise to life on Earth are widely represented in

various objects of our galaxy as well as in heavenly bodies that are located in areas of the Solar System close to us and that this evolution has proceeded quite far and has led to the abiogenic formation of diverse and complex organic substances, many of which play an important role in the life of contemporary organisms. But the problem of whether or not living bodies could develop on this basis still remains open. It can only be answered by the continuing penetration of man into space which is under way at the present time.

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DETECTION OF EXTRATERRESTRIAL LIFE

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ABSTRACT. The fundamentals are set forth of the evolution of chemical elements in the cosmos that led to the formation of various organic substances such as those uncovered in meteoritic carbonaceous chondrites. Possible extraterrestrial life forms are considered, along with the probability of presence of various groups of microorganisms on Mars. Requirements are examined that extraterrestrial life detection methods should fulfill, and some indirect life detection methods are reviewed. In particular, various cell proliferation detection methods are examined. Some of the design principles of automatic biological stations to be sent to the planets for extraterrestrial life detection are discussed.

Over the centuries, the problem of extraterrestrial life has been the province of philosophers, science fiction writers or scientists who solved it purely theoretically; this situation continued to exist until the 1950s. We must recognize, of course, that certain astronomical data, for example the seasonal variations on Mars and other observations, bore a relationship to this problem, but it was only indirect. In conjunction with the successes achieved in the study of space, this problem became one of those that lend themselves to solution by experimental methods. Naturally, the search for life on the planets will require the participation of representatives of the most diverse specialties. It soon became apparent that the detection of extraterrestrial life is a rather complex problem. The sending of stations to the planets in the Solar System is not one of those experiments which can be performed very frequently. This makes the position of the specialists more difficult, especially those engaged in the preparation of such experiments. One requires the most specific, reliable and carefully devised method or, what is better still, combinations of methods.

This article is devoted to a comparative survey of various methods that have been proposed for detection of extraterrestrial life. In this survey, we shall be using data from the literature as well as concepts based on the

results of studies in the field of space biology, performed under the direction of the author of this article.

Chemical Evolution

Some investigators feel that the detection of organic substances on the planets can serve as proof of the existence of life. It is impossible to agree with this for the following reasons.

1. The synthesis of various organic compounds has occurred and is still occurring in space. This is indicated by the fact that the meteorites (carbonaceous chondrites) that have fallen to Earth have been found to contain various hydrocarbons, fatty and aromatic acids, porphyrins and other organic substances. /28

2. It is assumed that certain substances, particularly ATP and ferroporphyrins, develop only as the result of biosynthesis. However, these and other substances may also be formed without the participation of living cells. The presence of porphyrins in petroleum and meteorites support this view.

3. The idea that the detection of optically active substances is proof of the existence of life also cannot withstand close examination. No one will deny that optical activity of observed substances makes their biogenic origin very likely. However, modern chemistry considers the possibility of abiogenic formation of optically active substances to be an absolutely certain possibility. In particular, the conditions under which meteorites are formed may be very suitable for this.

4. It is worth noting the fact that on the Moon, which does not have an atmosphere, all of the meteorites reach the surface. We can conclude that many carbonaceous chrondrites have fallen on the surface of the Moon over many millions of years. If the organic substances contained in them are not subject to decomposition, it is highly probable that they will be found in the lunar soil. Naturally, this will not prove the existence of life on the Moon.

5. The most significant proof that the detection of organic substances on a planet does not prove their biogenic origin must be considered to be the striking successes achieved in the field of organic synthesis. By using as energy sources factors which exist in space (ionizing radiation, ultraviolet

rays, electrical discharges, pressure), many investigators have succeeded in producing highly diverse organic substances from carbon dioxide, ammonia, hydrogen cyanide and other simple inorganic compounds: amino acids, carbohydrates, hydrocarbons, adenosine, etc.

Hence, the abiogenic synthesis of organic substances in space is undoubtedly a possiblity and consequently the detection of organic substances in the soil of the planets will not be proof of the existence of life upon them. This, of course, does not mean that a detailed chemical analysis of the soil of a planet will not be of extreme scientific interest. However, before these analyses are made, it is necessary to solve the problem of the existence of life on the planet by direct methods, which will be discussed below.

The Nature of Extraterrestrial Life /29

The latest data in the field of astrochemistry and astrophysics convince us of the fact that the same chemical elements and radicals are present in space as are found on Earth. The paths of chemical evolution in space, of which we spoke earlier, lead to the synthesis of compounds which are equivalent or very similar in nature to the organic substances which are found on our planet. The problem of the development of life on the planets cannot be treated outside of its connection with the origin of life on Earth. The opinion of outstanding scientists who feel that the precursors of life were chemoautotrophic microorganisms has not been split for a long time. The possibility of abiogenic biosynthesis of various organic substances on the planets makes it very likely that there is something to the idea that the initial forms of life were heterotrophic microorganisms. All of the above leads us to look on the planets for life whose chemical, energy and biosynthetic basis is similar to the basis of terrestrial life. Consequently, we must look for organisms whose composition includes carbon, nitrogen and phosphorus and in which the solvent is water. Of course, this does not mean that living conditions unlike those on Earth have not led in the process of evolution to the development of living matter that differs basically in its resistance to external factors and in the chemical nature of the metabolic processes from terrestrial forms of life. Usually the possibility of an

evolutionary path unlike that on Earth is not given sufficient consideration, as well as the creative role of selection, which is capable of forming on other planets substances that have highly unusual properties from the standpoint of inhabitants of Earth, and whose behavior and chemical metabolism are different as well. Therefore, it is completely logical to proceed on our search for life on other planets on the basis of existing data regarding life on Earth. It is necessary to look for life with a carbohydrate base and to be guided by modern data on ecology, physiology, biochemistry, microbiology and biophysics.

In the scientific respect, we are of course interested in those theories which deal with the possibility of life based on silicon or germanium, as well as a complete replacement in energetic processes of phosphorus compounds by sulphur compounds. In the case of planets with low soil temperatures, there is a possibility of existence of life in which the solvent is not water but ammonia or other compounds that remain liquid at low temperatures.

However, we can look for extraterrestrial life whose representatives do not contain carbon only in the event that numerous attempts to detect life on the planets which is based on carbon gives us systematic negative results. We cannot agree with the opinion of Lavlock, who does not consider it economically worthwhile to send spacecraft "... to detect a speculative life-form".

On the basis of the conditions existing on Mars, we can draw some conclusions regarding the existence of certain physiological groups of microorganisms in the soil of the planet. These data are presented below. Naturally, concepts of this kind have a purely hypothetical nature. /30

Microorganism	Existence
Photosynthetic bacteria	Highly probable
Chemosynthesizing bacteria	Possible, but oxidation not due to free oxygen
Heterotrophic microorganisms in the atmosphere	Not very likely
Heterotrophic microorganisms in the soil	Highly likely
Xerophytic microorganisms	Possible near the equator of the planet

Psychrophyllic microorganisms	Possible near the poles of the planet
Anaerobic microorganisms	Highly probable

Possibility of Detecting Extraterrestrial Life on Earth

The idea that extraterrestrial life is "imported" to Earth is very old. Many outstanding scientists and philosophers have accepted it. However, all experimental data thus far have not supported this hypothesis.

Much attention has been devoted to the content of living matter in meteorites. There are data available indicating that microorganisms have been found in meteorites. However, it was shown later that the meteorites which fell onto the soil of the Earth were rapidly contaminated by soil microflora and that only meteorites which have fallen in the Arctic, Antarctic and in deserts during the dry season are suitable for microbiological analyses.

In this regard, it is very interesting to perform a microbiological study of cosmic dust which falls on snow or ice in the Antarctic or Arctic and is collected far from meteorological stations.

It is completely possible from the theoretical standpoint that the spores of bacteria that are within a meteorite will not be killed by the action of ultraviolet rays, ionizing radiation or other factors existing in space. Less likely is the possibility that a living cell will be found in an anabiotic state in the course of many millions of years. In his Leeuwenhoeck lecture, Keylin gives examples of prolonged anabiosis, but it lasted only hundreds and several thousands of years. Could proteins exist for millions of years without undergoing denaturation? Extrapolation in this case is very difficult, but apparently this question must be answered in the negative. This is /31 supported in particular by the fact that the detection of viable water plants in salt deposits 200 to 250 million years old has not been supported by further studies. As far as the detection of inclusions in carbonaceous chondrites is concerned, reminescent of living substances and called on certain occasions "preprotobionts" and other names, it involves considerable difficulty.

The morphology of these bodies is so poor that there is no basis for drawing specific conclusions, and the various microchemical reactions are even less reliable, especially if we consider that carbonaceous chondrites contain various organic substances. These difficulties arise both in the study of meteorites and in attempts to find microbes in sections made from various ancient rocks.

Summing up what we have said above, we must reach the conclusion that representatives of extraterrestrial life have never been found on Earth.

Methods of Detecting Extraterrestrial Life

Requirements Imposed on the Method

All of the methods which have been suggested may be divided into two groups: direct methods and indirect methods of detecting extraterrestrial life. The latter includes highly diverse chemical analyses of the soil and atmosphere of a planet, astronomical methods and microscopy of the soil. The direct methods include transmission to Earth of "survey panoramas" and the proof of the growth and multiplication of single-celled organisms. The greater part of the researchers' attention should be devoted to the latter method, since only data obtained with its aid will be absolutely indisputable. Let us list the requirements which will be imposed on this method.

1. Optimum sensitivity, which means that life will not be discovered where there is none, and on the other hand, will be discovered where it exists.

2. The method must give reproducible results.

3. It must have the necessary reliability.

4. It must be based on the use of devices which are convenient in terms of weight and size.

5. It must be based on the use of only unquestionably sterile apparatus and reagents.

6. The method must not be based on analyses whose accuracy can be questioned, without knowing the chemical composition of the soil or the atmosphere of the planet on which the search for life is taking place.

7. The method must not be the only one. It would be most advantageous /32
to use several methods simultaneously which would confirm a single phenomenon or the same process.

Let us review briefly the existing methods for direct detection of extraterrestrial life.

Indirect Methods of Detecting Life

Astronomical methods. A number of existing observations bear direct relationship to the problem at hand. However, neither the seasonal variations on the surface of Mars, nor the absorption lines detected by Sinton, nor other observations of a similar nature, despite all of their interest and significance for science, can solve the problem of the existence of extraterrestrial life, especially on Mars. It is completely possible that when a scientific device circumnavigates the planet, carrying a telescope mounted aboard a spacecraft, it will be possible to obtain some new data in this area.

Microscopy. This method occupies an intermediate position between indirect and direct methods. By means of a microscope, it is possible to detect cells of microscopic animals or plants in a drop of liquid containing a particle of soil and what is more important, we can determine whether they are actively mobile. In this case, microscopy will be based on direct methods of observation. However, the preparation of such unstained preparations, the adjustment of the microscope setting, the possibility of a change in the chemical composition of the drop to inhibit Brownian motion, the need for microscopy at high magnification -- all of these make this method much more complex than the methods based on the confirmation of growth and multiplication of unicellular matter.

The preparation of fixed and stained preparations and their study under the microscope to detect microscopic water plants, fungi or animals has considerable possibilities. As far as bacteria are concerned, all of the experience of paleobotanists indicates that it is impossible to determine with

absolute certainty that a given spherical element is a fossilized cocciform bacterium. The more complex the morphology of the microorganisms, the greater the basis for hoping that the microscopic picture will be correctly interpreted. An important role may be played by luminescent microscopy, since the use of various luminophores will make it possible to detect the existence of nucleic acids, amino acids, etc. contained in the cells with a certain degree of reliability in differentiating living cells from dead ones, etc. The use of luminophores in combination with specific sera will make it possible to establish the membership of the detected microorganisms in a specific systematic form existing on Earth. Some consideration should also be given to the possibility of using a microscope to study the morphology of the cells in mixed cultures, grown with seeding of soil from the planet.

The use of electron microscopy involves familiar difficulties related to /33
the considerable weight of the electron microscope and the amount of electrical energy required to operate it. It is completely possible that miniaturization of the equipment will remove these obstacles.

Detection of Organic Substances

Earlier, when we were talking about chemical evolution in space, it was stated that the existence of organic substances on a planet cannot be an absolute proof of the existence of life on it. This in no way reduces the scientific significance of experiments involving the use of physical, physicochemical or chemical methods of analysis for the composition of the soil and the atmosphere of the planet. A quantitative determination of the porphyrins, flavins, amino acids, carbohydrates and many other compounds will give us very valuable results. The use of spectrophotometry, fluorometry, gas chromatography, mass spectrometry, polarimetry, and various analyzers for this purpose must naturally be included in the program for further studies. However, all of these methods are capable only of confirming the existence of these compounds, but cannot establish the relationship between their synthesis or decomposition, on the one hand, and the activity of living substances found in the soil of the planet on the other.

Direct Methods of Detecting Life

"Transmission of a panorama". Transmission of images of the surface of a planet, taken over a sufficiently long period of time and covering a certain area of the planet's surface, are undoubtedly worthy of our attention. Color transmission from a camera located on the planet would be especially valuable.

However, in the absence of sufficiently large sized animals or plants, the pictures that would be received would not give us the desired information. It would be more valuable to transmit pictures of the surface of the soil, taken at comparatively low magnifications.

Confirmation of Growth and Multiplication

The only and most reliable proof of the existence of extraterrestrial life must unquestionably be selected as the establishment of the ability of cells to reduplicate biopolymers. Only this method will make it possible to /34
follow a multiplication of cells with time, the assimilation of sources of energy and nutrition, as well as the origin of metabolism. This is not a static method, but unlike the isolated chemical determinations, is a dynamic one, and it must be given precedence over other methods. This method includes the collection of samples and their seeding, proof of the multiplication of microorganisms and transmission of the results to Earth. We will not discuss the latter.

Collection of samples. Many devices have been proposed for collecting samples of the soil of a planet. Without going into a discussion of the devices available for this purpose, we shall list the requirements which must be satisfied by the method of collecting samples: a) the collection of the soil sample must take place at some distance from the biological station on the surface of the planet; b) the amount of soil collected must be sufficient for seeding, and at the same time must not be excessive; c) the samples for seeding should not be collected from the deep-lying layers of soil and not from the surface either; d) the seeding material collected must not impede the observation of growth and multiplication of cells; e) collection of the atmosphere of the planet for microbiological analysis is less valuable than analysis of soil samples.

One of the successful methods of collecting samples is unquestionably the ejection of small sterile "shells" to a certain distance, to which a sticky tape or thread is attached. The soil particles that stick to them may then serve as a culture medium.

Nutrient medium. Proceeding from the ecological conditions existing on Mars, we can assume that we know what groups of microorganisms will be found on the planet. A list of the groups of these microorganisms will be found on pages 30 and 31. It is completely probable that the initial synthesis of organic matter is connected either with photosynthesis or with chemosynthesis, or with some other processes. In this case, there must be some heterotrophic bacteria, which break down organic matter, synthesized previously and located in the soil of the planet. This is precisely why it is advantageous to use nutrient media on which the largest number of forms of heterotrophic bacteria, found in the soil, will grow. A comparative estimate of nutrient media for this purpose may be accomplished by inoculating cultures with decreasing quantities of desert soil. The media in which the growth of soil bacteria is observed with seeding of the minimum quantity of desert soil will be worthy of further attention. The detection of heterotrophic microorganisms must be the first problem, since it is not only easier to accomplish than the detection of photosynthetic and chemosynthetic bacteria, but a theoretically larger quantity of heterotrophic microorganisms may be found in the soil of the planet.

Detection of Growth and Multiplication of Cells /35

The basis of this method is the determination of the growth and multiplication of microorganisms in a dynamic state. All of the indications that have been obtained with the aid of various methods immediately following inoculation may be interpreted as controls. Further recording of the changes must be made no earlier than 48 hours later. The periodicity with which the recording of the measurements is performed may be varied.

The accumulation of biomass may be evaluated on the basis of data from nephelometry or photometry. If these methods make it possible to establish the existence of from 10^3 to 10^4 cells per milliliter of culture, the methods

must be considered appropriate. The use of these methods requires conviction of the fact that during transportation of the nutrient medium by the spacecraft, the transparency of the medium did not change and that an overly large amount of soil was not added during inoculation, which could cause turbidity of the nutrient medium. This is precisely why the determination of the degree of turbidity of the nutrient medium is necessary immediately after inoculation with soil, in order to serve as a control.

The growth of a mixed culture, as a rule, is accompanied by a decrease in the oxidation-reduction potential of the culture fluid. Therefore, automatic determination of this potential of the culture as it grows can lead to valuable results. A slightly different type of information can be gained by determining the pH value of the culture, since the acidity can change differently depending on the composition of the nutrient medium and the physiology of the microorganisms that enter with the soil. It is completely valid to use media with glucose with a limited amount of sources of nitrogen nutrition. In this case, with inoculation using desert soil, one usually finds acidification of the medium and consequently potentiometry of the culture can also be included among the recommended methods, especially since it has already been proposed by outstanding specialists for this purpose.

During the development of microorganisms following seeding of the soil of a planet, decomposition of organic matter proceeds in parallel with the accumulation of cells. Carbon dioxide is given off regularly during fermentation and respiration, and hydrogen may also be given off during fermentation. When the chamber in which growth of microorganisms takes place is sealed, the pressure produced by the gases that are given off may be measured with the aid of a manometer. As the pressure increases, it may be recorded and the excess gas vented. Following a second accumulation of gas, another pressure measurement can be made. Hence, the method is a dynamic one. However, the manometric method is less promising than the use of nutrient media which contain organic substances with labelled carbon. The breakdown of these substances by microorganisms will be accompanied by the liberation of carbon /36
dioxide containing labelled carbon. A miniaturized counter will allow continuous recording of the amount of radioactive carbon dioxide given off. The

advantage of this method lies in the fact that various carbon-labelled substances can be included in the composition of the nutrient medium or else compounds in which various carbon atoms are labelled. It would be most interesting to use labelled protein, as well as labelled glucose and other substances. By using various labelled substances, it would be possible to get a rough idea of the physiological possibilities of microorganisms developing in the chamber.

Of course, it would also be interesting to study the decomposition of organic substances containing isotopes of sulphur. One of the isotope methods is that which is based on the fact that by using compounds enriched with O^{17} or O^{18}, the oxygen isotope in water can be detected as a result of the action of microorganisms on these compounds. As the substrate, investigators use phosphates, nitrates or sulfates, while the presence of the isotope is determined with the aid of mass spectrometry, which makes it possible to trace the enrichment of water with labelled oxygen. This method cannot be combined with the method described above for determining radioactive carbon dioxide for the following reasons: 1) partial exchange of oxygen ions may take place even without participation of the enzymatic systems of the cell; 2) there are no data on the amounts of O^{17} and O^{18} on the planets; 3) the labelled oxygen transferred from the anion to the water may combine with silicates; 4) exchange of ions is accomplished by enzyme systems which are less widespread among microorganisms than systems which participate in the breakdown of carbonaceous compounds, serving as a source of energy and carbon.

However, the method involving the use of oxygen isotopes is worthy of attention.

The growth of the biomass in the chamber in which seeding of the soil from the planet has been carried out can be measured as well on the basis of the accumulation of certain organic substances which are characteristic of the living cell. In particular, these substances include proteins which contain ferroporphyrins. Here, as in the other methods, it is important to establish that the increase in the amount of these proteins proceeds in parallel with the growth of the biomass. Quantitative determination of the ferroporphyrin proteins is accomplished by the chemiluminescent method, which

allows determination of the presence of hemin, catalase, peroxidase, hemoglobin, cytochrome c, etc. The system luminol + H_2O_2 reacts with a bright flash of chemiluminescence when catalysts are added to it to break up the hydrogen peroxide. The radiation detector is a photomultiplier, while recording is made with the aid of an oscillograph, galvanometer and strip-chart recorder. Chemiluminescence is at its maximum in an alkaline medium /37 (pH 11.0), so that a solution of caustic soda is added to the mixture of reagents. This is an important step, since the cells of the microorganisms are subjected to such changes in a strongly alkaline reaction that it becomes unnecessary to break them down, since the ferroporphyrin proteins are transferred to the culture liquid. The chemiluminescent method possesses sufficient sensitivity, and may be used to measure 10^{-9} gram/ml of catalase. It is necessary to keep in mind that the minerals which contain iron as well as the iron salts that are in the nutrient medium may produce a low constant background. This was the case in particular for the introduction of ground limonite to the reaction vessel. However, this slight background does not disturb the subsequent determinations. The increase in biomass can also be measured by the accumulation of the enzyme phosphatase in the culture, as determined by the fluorescent method. It is also possible to measure the amount of flavins contained in the culture by determining the increase in the mixed culture following seeding with the soil from the planet.

Completely justified attention has been attracted by a method based on the quantitative determination of ATP, contained in the cells of multiplying microorganisms. It is based on the phenomenon of bioluminescence, in which the role of the energy substrate is played by luciferin and luciferase serves as the catalyst and simultaneously as the scintillation agent. For this reaction, it is necessary to have both ions of Mg^{++} and oxygen. In the presence of ATP, a process involving the emission of light begins, which can be recorded by using the same apparatus as for determining the ferroporphyrin proteins by the chemiluminescent method. The sensitivity of the "firefly" method is quite high; this method can be used to determine from 10^{-9} to 10^{-11} gram/ml of ATP, so that it has been recognized as one of the methods that may be recommended for detecting extraterrestrial life. Unfortunately, this method

can only be used in the event that the cells of the microorganisms are broken down beforehand. The use of an ultrasonic or mechanical disintergrator for this purpose is highly complicated, since it increases the weight of the apparatus and calls for additional electrical energy. This difficulty may be overcome by using various enzymatic preparations obtained from cultures of microorganisms and possessing the ability to dissolve the cell wall of microorganisms. Good results in this direction are also produced by lysozyme.

The multiplication of microorganisms is regularly accompanied by the accumulation of optically active substances in the culture. The dynamics of their accumulation may be determined with the aid of a polarimeter or a spectrophotopolarimeter and are worthy of attention, since in this case the optically active substances are formed as the result of biosynthesis.

It is also possible to use devices which measure the increase in the /38
temperature of the culture liquid itself, as is seen in the case of the multiplication of cells in Dewar vessels or chambers equipped with heat insulation. During the intensive development of the culture and the accumulation of a significant biomass, the calorimetric method may be employed successfully. However, it is quite possible that the growth of the microorganisms in a liquid nutrient medium will not be so dramatic in nature, in any case, not until optimum conditions have been determined for such development. However, a small degree of multiplication among the cells may not give off a significant amount of heat.

The methods that are used for detecting extraterrestrial life may be subjected to comparative evaluation and divided as a result into three groups: the most promising methods, suitable ones and those whose use is less promising. This classification of methods and analytical procedures is given below.

Analytical Procedures Proposed for Detecting Extraterrestrial Life

Most Promising	Worthy of Attention	Less Promising
Determination of the growth of the biomass.	Determination of optical activity.	Use of O^{17} and O^{18}.
Quantitative determination of ferroporphyrin proteins (in dynamics)	Quantitative determination of flavins.	Use of an isotope of sulphur.
Quantitative determination of ATP (in dynamics)	Quantitative determination of protein, nucleic acids and amino acids.	Calorimetry (determination of the amount of heat formed during the growth of microorganisms).
Quantitative determination of emitted radioactive carbon dioxide.	Determination of phosphatase activity.	Determination of mitogenetic radiation.
Measurement of the pH of the culture. Measurement of the Eh of the culture.	Manometry (determination of the pressure in the chamber containing the culture).	

These data do not pretend to be exhaustive, but they are based on the best-known recommendations.

High-Speed Methods of Detecting Extraterrestrial Life

These methods cannot be based on seeding of soil and subsequent recording of changes taking place in a culture of fluid. This excludes the possibility of determining the dynamics of the changes which ensures reliability. It is impossible to use isolated, short analyses of the soil of a planet to obtain completely reliable proof of the existence of life. However, the performance /39 of a number of functional tests with the soil of the planet may be of definite interest. Naturally, in those analyses which last one to two hours, it is necessary to examine soil samples that are significantly larger in size and weight than those which serve as the seeding material in experiments involving the seeding of the soil in a nutrient medium.

As was established in our laboratory, it is possible with the aid of isolated short analyses to examine desert soil and find ferroporphyrin proteins, ATP and to establish the capacity of soil samples to cause breakdown of

glucose containing labelled carbon and the liberation of radioactive carbon dioxide by virtue of the microflora contained in it. However, it is also possible to use other methods of detecting extraterrestrial life described in the literature for this purpose. However, the high-speed methods of determination, as we have already pointed out, are much less reliable than those methods which allow the growth and multiplication of cells of microorganisms to be determined.

Some Principles for the Design of Biological Stations

The construction of apparatus for detecting extraterrestrial life goes beyond the limits of competence of biologists. However, certain views must be expressed which are based on the study of various methods of detecting extraterrestrial life. First of all, it should be pointed out that the recording of the growth and multiplication of cells which must be carried out following the seeding of the soil of the planet must be performed simultaneously with the aid of several methods. Their number may vary from three to six. The selected methods must be based on the recording of various phenomena or processes whose increase unquestionably indicates the growth and multiplication of cells. In the majority of cases, repeated obtaining of information may be carried out with a single mixed culture, for example, determination of the number of cells or determination in the medium (on the basis of the growth of the culture) of the amount of physiologically active substances. However, in those cases when destruction of the cells is necessary for analysis, there must be a number of vessels for growing cells. The destruction of the cells and the analyses are performed consecutively at various intervals, and therefore repeated use of the culture is precluded. If the same recording devices may be used for obtaining information regarding different processes (for example, photomultiplier and the determination of the intensity of luminescence), it is completely justifiable to use this kind of recording device while the chambers in which the microorganisms develop remain fixed. As we know, the temperature of the surface of a planet, Mars in particular, varies within rather wide limits. However, it would be incorrect to create temperature variations /40
in the chamber used for growing the bacteria. On the Earth, all psychrophyllic bacteria do not lose the ability to multiply intensively (much more

rapidly than at low temperatures) at higher temperatures. Therefore, it is necessary to maintain at all times a temperature of 20-30° in the chamber. The number of occasions on which recording of the changes taking place as a result of the growth of the microorganisms in the chamber can be performed must be equal to a minimum of three, but it would be desireable to perform them on more occasions.

All of the apparatus in the biological station must perform tests which ensure that neither the vibrations nor the launching or landing of the spacecraft have any effect on the operation of the apparatus. All of the reagents, nutrient media, etc. must not change their characteristics during the long flight and must preserve their sterility.

The results of the determinations may be transmitted at once to Earth, but obviously their storage in a memory device with subsequent transmission of all information would be more efficient.

It is completely possible that even before a biological laboratory lands on a planet of the Solar System, Mars in particular, our information regarding the conditions existing there may be expanded. This requires making changes both in our concept of the existence of extraterrestrial life and in the design of instruments. In this article, we have discussed the most reliable method of detecting extraterrestrial life, based on the measurement of the dynamics of the growth and development of heterotrophic microorganisms, which may be found in the soil of a planet. However, their existence is possible in only two cases. Either various organic substances that arose as a result of chemical evolution are present in sufficient amounts on the planet, i.e., as the result of abiogenic synthesis, or else chemo- or photosynthetic microorganisms exist on the planet and synthesize organic substances from the carbon dioxide in the atmosphere. Obviously the second possibility is the more likely one. Therefore, the next stage in the search for life will involve attempts to find photosynthesizing and chemosynthesizing organisms in the soil of the planet. Naturally, in order to detect them it will be necessary to modify the methods that are used for growing heterotrophic microscopic materials. However, these changes will not be fundamental, since the

ability to synthesize the substances composing the body from carbon dioxide may be established in various ways.

In searching for extraterrestrial life, we may encounter two interesting possibilities. First of all, life may be missing at the present time, but soil analyses will show that there are traces of former life as indicated by morphological and chemical studies. Hence, provision must be made in the program for paleobotanical and paleozoological studies. The second possibility consists in the fact that it will not be microorganisms that are found on the planet (since they are already quite complexly organized substances in an evolutionary sense) but primitive life forms that represent the very start of evolution. The discovery of such primitive life forms will naturally be of extremely great interest, even more than the discovery of organisms resembling terrestrial ones. /41

However, there are as yet no methods with which such "precursors of life" could be detected. This does not mean, of course, that such methods will not be used in the future.

NUTRIENT MEDIA APPLICABLE TO MICROORGANISM DETECTION ON MARS

L. A. Kuzyurina and V. M. Yakshina

ABSTRACT. A selection was performed of the optimal mineral-organic medium for the growth of soil microorganisms extracted from the desert soils of Africa and Karakum, arid gray soil of Pamir, sandy soil of Alma Ata, and red soil of Georgia. It is assumed that life conditions in these soils bear some remote resemblance with those in Martian soils.

The sending of biological stations to the planets in the Solar System has become a reality. In conjunction with this, many questions arise that relate to the methods of detecting extraterrestrial life, the design of biological stations, etc. Principal attention is devoted to the possibility of detecting microorganisms on other planets, especially Mars, and this naturally has led in turn to the raising of the question of the necessary nature for the nutrient medium in which the seeding of the samples collected on the planet is to take place. This article is devoted to a comparative evaluation of various nutrient media.

We know that the air on Mars contains very little water vapor. Kuiper (1951) found traces of water vapor in the infrared spectrum of the "maria" and also detected its absorption in the ice of the polar caps.

Firsov (1966) suggests that with the low barometric pressure and low water vapor content in the atmosphere, the absorption of water by the rocks on the Martian surface may be a very important ecological factor. The author suggests that the water table on Mars is at a subsoil level and as a rule the water does not emerge on the surface. Vokuler (1956) believes that the climate on Mars does not differ markedly from that on Earth and is only slightly more severe.

A number of scientists (Tikhov, 1953, Lyubarskiy, 1962; Imshenetskiy, 1962; Oparin, 1966; Fesenkov, 1966; Firsov, 1966, et al.) suggest that life on Mars has a carbonaceous nature and that water serves as the solvent. Oparin and Fesenkov (1960) write that we must exclude from scientific consideration /42

all kinds of fantasies about quartz organisms and similar "fire beings". Oparin (1966) suggests that the evolution of life on Mars may have taken place in the same way as on Earth, and if Mars was formerly richer in water, as many modern authors believe, the high ability of organisms to adapt to new conditions of their environment makes it possible that living matter has adapted to the bleak Martian climate.

Levin et al., (1962) put together a fluid nutrient medium containing carbon-labelled compounds which was sent with the "Gulliver" apparatus to detect Martian microorganisms. If they exist in particles of soil that are collected, their multiplication on the nutrient medium will liberate radioactive carbon dioxide gas. Information regarding the amount of carbon dioxide liberated in this dynamic process will be transmitted to Earth.

The authors tested nutrient media with cultures of various bacteria, actinomycetes, fungi and water plants and decided on a medium with the following composition (in grams): K_2HPO_4 -- 1.0; KNO_3 -- 0.5; $MgSO_4 \cdot 7H_2$) -- 0.2; NaCl -- 0.1; $FeCl_3$ -- 0.01, amino-acid hydrolysate -- 4.0; yeast extract -- 13.0; soil extract -- 250 ml; proteose peptone -- 20.0; malt extract -- 3.0; ascorbic acid -- 0.2; l-cysteine -- 0.7; meat extract -- 3.0; distilled water -- up to 1 liter. The authors proceeded on the basis of an assumption that the Martian microorganisms do not differ substantially in their physiological and biochemical characteristics from terrestrial forms and are able to use the necessary sources of nutrition in a medium rich in nutrient substances. From among the organic compounds containing radioactive carbon C^{14}, they tested glucose, formic acid, sodium acetate, sodium pyruvate, glycine and yeast extract, as well as cysteine, containing radioactive sulphur S^{35}. In experiments with *Escherichia coli*, the best results were obtained using labelled formic acid or glucose with an activity equal to 5 microcuries per 1 ml of medium. The control chamber contained everything that was contained in the experimental chamber, but antimetabolite was added to it as well. The test of the operation of the device under terrestrial conditions was successful.

In addition to the heterotrophic microorganisms on other planets, especially Mars, there may be photoautotrophs and chemoautotrophs. However, the

search for heterotrophs must be given primary consideration. In our tests, we selected media which can be used to detect the growth of microorganisms when seeded with minimum quantities of soil.

Experimental Section /43

Method

The purpose of our work was the selection of a transparent (very important from the standpoint of photometry or nephelometry) nutrient medium, suitable for the growth of the majority of heterotrophic soil microorganisms that are encountered in arid soils, whose conditions may be similar to some degree to those on Mars (sharp diurnal temperature variations, dryness of the soil, low content of organic substances).

The following soils were collected: from the Karakum Desert (the Repeteke and Darvazy rayons), arid serozem soil (Pamir), soil from the Nubian Desert (Central Africa, Martsut region, Province of Takhrir), with a depth of 1 m[1], krasnozem (Georgian SSR), and sandy soil (Alma-Ata).

The soil was added to fixed media: a modified synthetic Rana medium, No. 2, 3, 4, 7 and 11, as well as on potato-peptone broth (PPB) with phosphate buffer without chalk and meat-peptone broth (MPB). Data on the composition of the nutrient media are listed in the table.

As the carbon source, we used glucose; in addition to the latter, in three media we also used sodium pyruvate. Ammonium nitrate, ammonium chloride or ammonium phosphate were used as nitrogen sources, as well as asparagine. Liver and yeast extracts and a mixture of microelements were added to certain media.

A mixture of microelements with the following composition (in g/l) was added to five organic-mineral media: distilled water -- 1 liter, $Al_2(SO_4)_3$ -- 0.3; H_3BO_3 -- 5.0; $(NH_4)_2MoO_4$ -- 5.0; KI -- 0.5; KBr -- 0.3; $ZnSO_4$ -- 0.2;

[1] We would like to thank T. B. Kazapskiy for supplying us the soil sample from the Nubian Desert.

$MnSO_4$ -- 0.08; $Co(NO_3)_2$ -- 0.1; $FeCl_3$ -- 0.01. To each liter of nutrient medium, we added 1 milliliter of this mixture of microelements. Experiments were performed in sets of three in two parallel tests at a temperature of 28°.

All of the media had a pH of 6.8-7.2 (phosphate buffer was added).

It was necessary to establish in which nutrient media the growth of microorganisms took place when minimum quantities of seeding material were added. One gram of soil (converted to absolute dry weight) was added to the first test tube containing 9 ml of a certain nutrient medium and then successive dilutions were performed with this same medium up to 10^{-15}. Calculation of the amount of microorganisms in the soil samples when added to liquid nutrient media were performed by the method of maximum dilutions and subsequent evaluation of the results with the aid of the Macready variation-statistical table (Aristovskaya et al., 1962). The best nutrient medium was judged to be the one in which growth developed after seeding with the maximum dilution of the soil mix. /44

Results of Investigations

The figure shows the increase of microorganisms in liquid nutrient media following seeding with various soils. It follows from the diagram that at the very highest dilutions of the soil, growth is detected on mineral-organic media 11 and 7. On medium 11, Pamir serozem, krasnozem and sandy soil from Alma-Ata had a maximum titer of 10^{10}, while the soil from the Karakum Desert has values of 10^8 and 10^5, but in comparison to the growth of microorganisms from these soils on other media, the titers on medium 11 remained highest. The limiting titers for soil microorganisms on medium 7 was somewhat lower (on the order of 10^7), with the exception of the krasnozem.

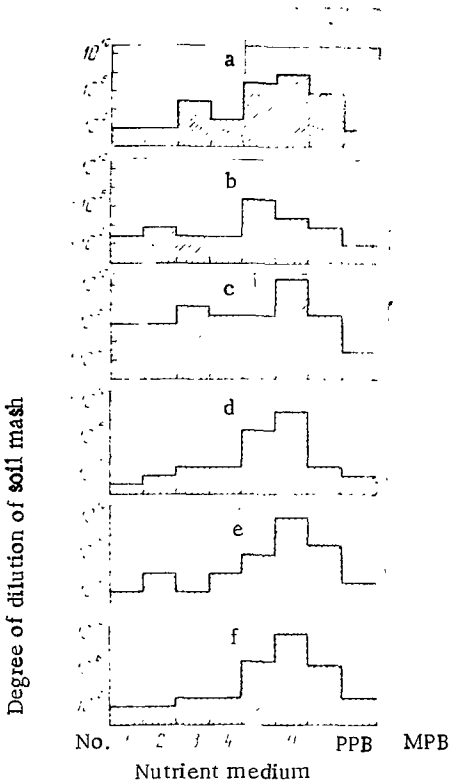
In its nutrient properties, potato-peptone broth resembles mineral-organic medium 7, judging by the maximum titer of soil microorganisms. The titer of the microbes on the potato-peptone broth was somewhat less than on medium 7. /45

COMPOSITION OF LIQUID NUTRIENT MEDIA (IN GRAMS/LITER OF DISTILLED WATER)

Component of the Medium	Altered Rana Medium	Number of Medium					PPB*
		2	3	4	7	11	
Glucose	10	10	10	10	10	10	--
Asparagine	--	--	--	1	0.7	0.1	--
Sodium Pyruvate	--	--	2	2	2	--	--
NH_4Cl	--	1.0	1.0	--	0.3	--	--
NH_4NO_3	--	--	--	--	--	0.5	--
$(NH_4)_2HPO_4$	5	--	--	--	--	--	--
K_2HPO_4	5	2.61	2.61	2.61	2.61	2.61	--
KH_2PO_4	--	0.91	0.91	0.91	0.91	0.91	--
KCl	1	--	--	--	--	--	--
$MgSO_4\ 7H_2O$	0.1	0.3	0.3	0.3	0.3	0.3	--
$CaCl_2$	--	0.1	0.1	0.1	0.1	0.01	--
NaCl	Traces	0.1	0.1	0.1	0.1	0.1	--
$FeCl_3$	"	--	--	--	--	--	--
Mixture of microelements	--	1 ml	1 ml	1 ml	1 ml	1 ml	--
Yeast autolysate (6% substance in water)	--	--	--	--	--	5 ml	--
Liver extract (4.8% substance in water)	--	--	--	--	1 ml	1 ml	--
pH of the medium	7.0 7.2	6.98	7.0	6.98	6.98	6.9 7.0	6.96

*20% potato broth with 0.5% peptone.

Media 11 and 7 are similar in their composition. They have the same mineral composition, mixture of microelements and added amounts of yeast and liver autolysates. Medium 7, in addition to glucose, contains sodium pyruvate. The nitrogen sources in both media are asparagine and ammonium salts, but medium 11 also contains nitrate as well. It is quite possible that the presence of sodium pyruvate is not so important in the medium when glucose is present as well, as the diversity of the sources of nitrogen nutrition:



Growth of Microorganisms On Liquid Nutrient Media Following Seeding with Various Soils.

a, Soil from Karakum Desert (Repetek); b, Soil from Karakum Desert (Darvaza); c, Pamir serozem, arid soil; d, Soil from Nubian Desert; e, Krasnozem (Georgian SSR); f, Sandy soil (bright serozem, Alma-Ata). The axis of ordinates is negative.

asparagine, ammonium and nitrate salts. Hence, as a result of the comparison of the degree of suitability of the nutrient media for the growth of heterotrophic microorganisms, found in desert soils, medium 11 was found to be best.

It is important to note that the titer of soil microorganisms in the meat-peptone broth did not exceed 10^3, regardless of the nature of the desert soils with which it was inoculated. At the same time, however, the titer on medium 11 was higher by six to seven orders of magnitude. A different picture was seen when chernozem was used for seeding. On the medium 11 and on MPB, the titers were higher and the maximum titer on MPB exceeded the titer on medium 11 by one order of magnitude.

It was confirmed experimentally that the best medium (11) was suitable for growing the most diverse microorganisms upon it. Thus, after 18 hours at a temperature of 28°, *Bacillus megaterium* will grow following seeding from a dilution of 10^{-5}. while *B. prodigiosum* will develop from 10^{-12}. On an agarinized medium 11, the following microorganisms will grow well: *B. fluorescens, Escherichia coli, B. prodigiosum, Seliberia stellata*, strain 2, nov. sp. (isolated by Aristovska), *B. mesentericus, B. cereus, B. megaterium, B. simplex, B. cylindrosporus*, psychrophyllic actinomycetes No. 921 and No. 924, isolated by Korenyako, *Actinomyces violaceus, Mycococcus oligonitrophilus, Mycobacterium roseo-album* /46

strain 368 (nitrogen fixer), isolated by Kalininska; *Saccharomyces cerevisiae, Zygosaccharomyces thermotolerans, Pichia vanriji, Zygowilliopsis californicus, Hanseniaspora javanica, Aspergillus niger.*

It should be kept in mind that recently a number of new forms of microorganisms have been isolated from soil (Nikitin et al., 1966). Some of these cultures[1] have been seeded on medium 11, and it was also found that *Agrobacterium polyspheroidum* also grows on this medium.

In conclusion, we would like to express our profound appreciation to Academician A. A. Imshenetskiy for directing the work.

Conclusions

1. Soil microorganisms have much higher growth titers on mineral-organic nutrient media with yeast and liver extracts and a mixture of microelements than on modified Rana medium.

2. Medium 11 proved to be the best nutrient medium. When the following soils were seeded on this medium, the highest growth titer in soil microorganisms was observed: Nubian Desert (Africa) and Karakum Desert (Tadzhikistan), arid serozem (Pamir), sandy soil (Alma-Ata) and krasnozem (Georgian SSR). The exception was found in the case of seeding made with soil from the Karakum Desert (Darvaza): on medium 7, growth of microorganisms was found following seeding from a high dilution.

3. On mineral-organic media which do not contain liver or yeast extracts and microelements, seeding with all of the studied soil samples was followed by a determination of the lowest titer of soil microorganisms.

4, The most suitable medium for growing soil microorganisms in the seeding of desert soils was meat-peptone broth: the titer of bacteria did not exceed 10^3 and on medium 11 the titer was six to seven orders of magnitude higher.

[1] We express our deep appreciation to D. I. Nikitin for supplying us with new forms of soil microorganisms.

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USE OF PHOTOMETRY IN THE ULTRAVIOLET AND VISIBLE REGIONS FOR THE RECORDING OF THE GROWTH OF MICROORGANISMS IN A LIQUID MEDIUM

G. G. Sotnikov

ABSTRACT: The possibility of using the photometric method to detect signs of extraterrestrial life on the planet Mars is discussed. It is shown that the background from limonite, in the amount of 2.5 g/liter of transparent nutrient medium, can make the performance of photometric measurements difficult. When measuring absorption in the ultraviolet region at λ = 280 nm, the process of multiplication of microorganisms shows up most clearly. Photometry in the ultraviolet and visible regions of the spectrum makes it possible to determine the rate of multiplication of microorganisms in dynamic form and to confirm their death, characterized by a decrease in optical density.

One of the most characteristic properties of microorganisms is their ability to absorb light of certain wavelengths in the ultraviolet region.

As we know, the various components of the microbe cell, such as the amino acids, proteins and nucleic acids, have a completely determined maximum of absorption in the ultraviolet region. The maximum absorption for protein lies in the region of 280 nm while that for nucleic acids is 260-265 nm.

When light in the visible portion of the spectrum passes through a suspension of microorganisms, we see essentially a scattering of the light by the bacteria, which depends on the concentration, size and coefficients of refraction of the cells (Fikhman, Petukhov, 1967). Absorption of light in the visible portion of the spectrum by intracellular pigments is insignificant. With the aid of modern spectrophotometers, it is possible to measure directly in the suspension only the absorption spectra of the cytochromes, which have /48 rather narrow and characteristic absorption bands (Lisenkova, Mokhova, 1967). It should be mentioned that the sensitivity of modern optical apparatus, recording the scattering of light by a suspension of microorganisms, amounts to 10^6 cells per milliliter for a unicellular green water plant (*Chlorella*) measuring 3-5 microns (Filippovskiy et al., 1967; Brandt, 1967). In the case of bacterial cells, insignificant changes in the optical density of suspensions containing from $5 \cdot 10^7$ to $5 \cdot 10^9$ cells per milliliter have been reliably

recorded (Sid'ko et al., 1967). By using the Vishniac method (Vishniac, 1960), the American engineers Buckendahl, Ried and Lemberg (1965) designed a nephelometer with an ingenious optical system for recording the multiplication of microorganisms on the basis of the scattering of light. The minimum concentration that can be detected by this device mounted in a "Wolf trap" intended for detecting extraterrestrial life is equal to 10^4 cells per milliliter.

Experimental Section

Method

To measure the total absorption and scattering of light in the ultraviolet and visible regions of the spectrum by the cells of microorganisms multiplying in a nutrient medium, a device based on the SF-4 spectrophotometer was built. In order to increase sensitivity of this device, the F-4 and F-5 photocells were replaced by photomultipliers (FEU-39) for the ultraviolet region and FEU-42 for the visible. To record the signal variations, a high-resistance electronic strip-chart recording potentiometer EPPV-60MZ was used (Demidova et al., 1967). The noise level in the potentiometer was reduced by means of the selective properties of the amplifier, equipped with interstage negative feedback.

The diagram of the modified photometer for use in the ultraviolet and visible ranges is shown in Figure 1.

The operating principle is as follows: light from the lamp passes through a monochromator, making it possible to work at the fixed wavelength, and then enters a quartz cell containing the suspension of microorganisms to be studied; the light is absorbed, scattered and then strikes the light sensor, a photomultiplier, where the light signal is transformed into an electrical one, amplified and then recorded by the electronic strip chart recording device for measurement and recording of small currents ($5 \cdot 10^{-7}$-$5 \cdot 10^{-11}$ ampere). There was practically no zero drift. The operating voltage of the FEU-39 is 1300 volts. The working voltage on the FEU-42 is 1000 volts.

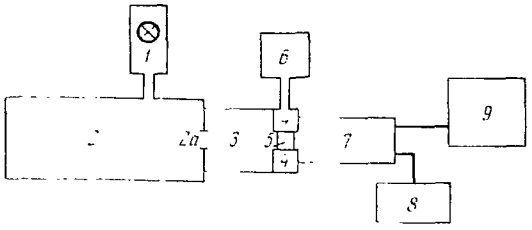

Figure 1. Diagram of Modified Photometer For Working in the Ultraviolet and Visible Regions of the Spectrum.

1, Hydrogen lamp for working in the spectral range from 220-320 nm or incandescent lamp for the visible region; 2, Quartz monochromator of SF-4A spectrophotometer; 2a, Exit slit of monochromator; 3, Cell section of the spectrophotometer; 4, Temperature-stabilized cell; 5, Quartz cell with capacity of 5 ml (5 mm wide); 6, Ultrathermostat; 7, Radiation sensor-photoelectronic multiplier; 8, High-voltage stabilized rectifier; VS-22; 9, Electronic strip-chart recording device for measuring and recording small current, EPPV-60MZ.

We begin by testing the sensitivity of the instrument. The photometer records the concentrations of protein up to 10^{-8} g/ml on the basis of absorption at a wavelength of 280 nm, and determines the concentration of yeast cells (*Saccharomyces cerevisiae*) in amounts up to $5 \cdot 10^3$ cells per ml of physiological solution and spores of *Bacillus stearothermophilus* (strain 214) in the amount of $2 \cdot 10^5$ spores per ml of mineral medium.[1]

To obtain spores, *Bacillus stearothermophilus* (strain 214) was grown on tomato agar at 55° for 10 days.

The signal from the absorption of the physiological solution, the nutrient medium, was assumed to be zero absorption in relative units with monochromatic excitation at 280 nm. The error of the method did not exceed 2%.

Later, we studied soil from the Karakum Desert (Repetek), which is relatively poorer in microflora, and continuously cultivated garden soil from the middle latitudes of the European portion of the USSR. Measurement of

[1] The composition of the medium (in g/l) was as follows: K_2HPO, 30; KH_2PO_4, 10; NH_4Cl, 5; Na_2SO_4, 1; $MgSO_4 \cdot 7H_2O$, 0.1; $MnSO_4$, 0.1; $FeSO_4 \cdot 7H_2O$, 0.1; $CaCl_2$, 0.001; pH 7.3.

absorption and scattering by the microorganisms was performed at different wavelengths: 280, 380, 480 and 680 nm. Suspensions of soil equal to 5 mg were added to 10 ml of nutrient medium. The initial signal from the prepared suspension of microorganisms from desert soil in the medium was assumed to be zero absorption and the scattering was expressed in relative units with monochromatic excitation. The initial concentration of microorganisms in spores was $1 \cdot 10^3$ cells per milliliter. The number of cells was estimated in a Goryayev chamber. Experiments were performed at 27, 37 and 47°. /50

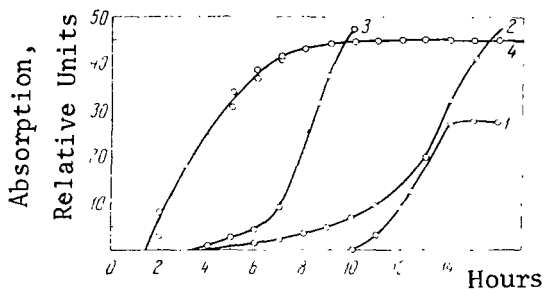

Figure 2. Measurement of Absorption With Monochromatic Excitation, λ = 280 nm, During the Process of Multiplication of Microflora from Desert Soil at Various Temperatures.

1, At 27°; 2, at 7°; 3, at 37°, with the soil moistened beforehand at 4°; 4, at 47°.

In the controls, 0.5% formalin was added to the medium, which did not introduce any significant changes in the absorption and scattering, but stopped the process of multiplication of the microorganisms. The control signal at the beginning and end of the experiment was constant.

In Figure 2, we have plotted the curves showing the dependence of absorption on time in relative units at 280 nm. Curve 1 shows the relationship of the absorption of light during the process of multiplication of microorganisms with time at 27°. The signal was recorded only after 11 hours. The final concentration was equal to $1 \cdot 10^8$ cells per milliliter.

At 37°, the signal was recorded after 5-6 hours (curve 2). When using desert soil previously moistened for a day at 4° for the purpose of seeding, development proceeds more rapidly and reaches a stationary concentration of $1 \cdot 10^9$ cells per milliliter in 10 hours (curve 3). In seeding with unmoistened desert soil, the stationary concentration equal to $1 \cdot 10^9$ cells per milliliter was reached in 16 hours (curve 2).

At 47°, the signal is recorded after 2-3 hours (curve 4). One can clearly see the different nature of the curve showing the dependence of

absorption on time. It is precisely during the first hours (up to 5 hours) that the greatest amount of slope (about 60°) is seen in the curve, indicating a low value for the generation time, i.e., a high rate of multiplication of the microorganisms. Later, the multiplication process slows down by a factor of two.

The purpose of this study was primarily to determine the possibility of using photometric methods to determine whether there are any signs of life on the planet Mars. The study of the polarization of light reflected from Mars suggests that its surface is largely covered by limonite (Vokuler, 1956). It was necessary to determine the degree to which the background from limonite would hinder performance of photometric measurements. It was found that when limonite is added in amounts greater than 2.5 g/l of transparent nutrient /52
medium, it becomes impossible to obtain a positive signal due to the multiplying microorganisms.

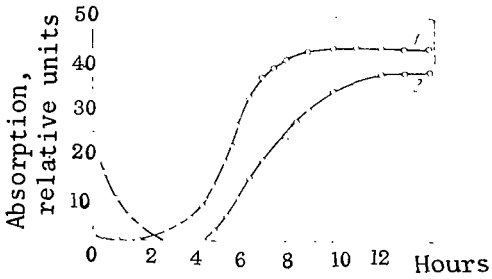

Figure 3. Change in Absorption of Monochromatic Light at λ = 280 nm During the Process of Multiplication of Microflora from Desert Soil (at 37°).

1, 2.5 mg soil + 2.5 mg limonite per 10 ml of medium;
2, 25 mg of soil + 25 mg limonite per 10 ml of medium.

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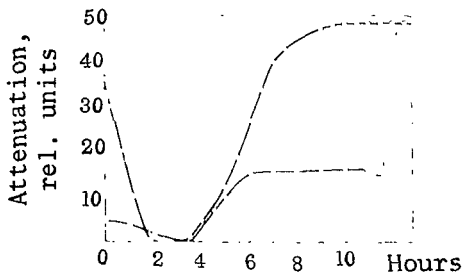

Figure 4. Change in Attenuation of Monochromatic Light at λ = 480 nm During the Process of Multiplication of Microflora from Desert Soil (at 37°).

1, 2.5 mg soil + 2.5 mg limonite per 10 ml of medium;
2, 25 mg of soil + 25 mg limonite per 10 ml of medium.

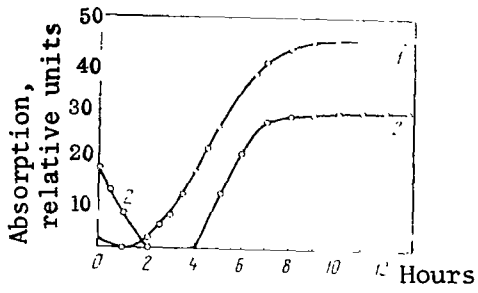

Figure 5. Change in Absorption of Monochromatic Light at λ = 280 nm During Multiplication of Microorganisms Following Seeding of Soil from the central Latitudes of the European part of the USSR (at 37°).

1, 5 mg of soil per 10 ml of medium; 2, 50 mg of soil per 10 ml of medium; initial concentration equals $2 \cdot 10^6$ cells per milliliter; stationary concentration equals $1.3 \cdot 10^{99}$ cells per milliliter.

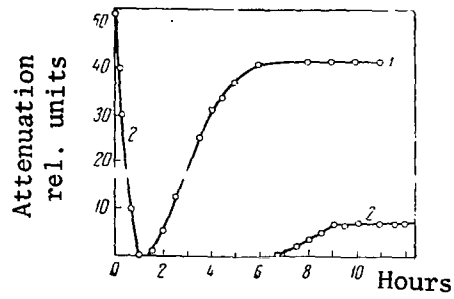

Figure 6. Change in Attenuation of Monochromatic Light at λ = 480 nm During Multiplication of Microorganisms Following Seeding of Soil from the Central Latitudes of the European Part of the USSR (at 37°).

1, 5 mg of soil per 10 ml of medium; 2, 50 mg of soil per 10 ml of medium; initial concentration equals $1.5 \cdot 10^6$ cells per milliliter; stationary concentration equals $1 \cdot 10^9$ cells per milliliter.

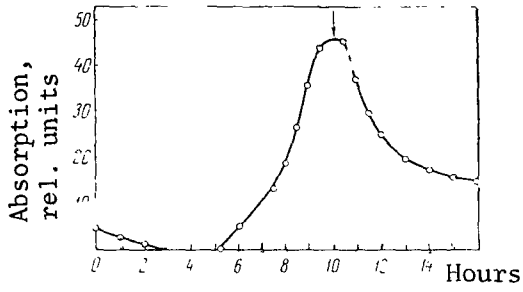

Figure 7. Change in Absorption of Monochromatic Light at λ = 280 nm During Multiplication and Dying Out of Microorganisms Following Seeding With Sandy Soil (at 37°); 1 mg of desert soil per 10 ml of medium.

The arrow indicates the moment at which formalin was added.

We can see from Figures 3 and 4 that with a concentration of desert soil and limonite equal to 2.5 mg per 10 ml of nutrient medium the precipitation of mineral particles to the bottom of the quartz cell takes place quite rapidly (in 2-3 hours) and the initial background is insignificant (2-4 relative units). The optical density for the nutrient medium, measured with the aid of the FEK-56 with a red light filter No 9 equals 0.02. For 5 mg of limonite in 10 ml of nutrient

medium, the optical density is equal to 0.06. When the limonite concentration is increased by a factor of 10, the optical density increases accordingly by an order of magnitude (0.63-0.75).

With a total content of limonite and sand of 50 mg (in a ratio of 1:1) per 10 ml of medium, the initial turbidity increases by a factor of 9-10 and in 2-3 hours there is complete precipitation of the inorganic particles under the influence of the force of gravity. Since the force of gravity on Mars is 0.38 g of that on Earth, the process of precipitation of particles will proceed more slowly, which will have a negative effect on the obtaining of a useful signal. Completely analogous curves were obtained for soils rich in organic matter and microflora. We can see from Figures 5 and 6 how the precipitation of mineral particles proceeds and how the process of multiplication of microorganisms takes place.

It is necessary to point out an important detail in this connection: when measuring absorption at 280 nm, the process of multiplication of microorganisms becomes more definite (Figure 3 and 5, curve 2). Upon the death of the microorganisms which are in a stationary phase of development, there is a decrease in the optical density of the culture medium. After 16 hours, the useful signal decreases by a factor of 3 (Figure 7).

Conclusions /53

1. Photometry in the ultraviolet and visible regions of the spectrum makes it possible to observe the multiplication of microorganisms in a dynamic fashion.

2. Working concentrations determined by the photometer in the ultraviolet range equal $n \cdot 10^5$ cells per milliliter, and $n \cdot 10^6$ cells per milliliter in the visible.

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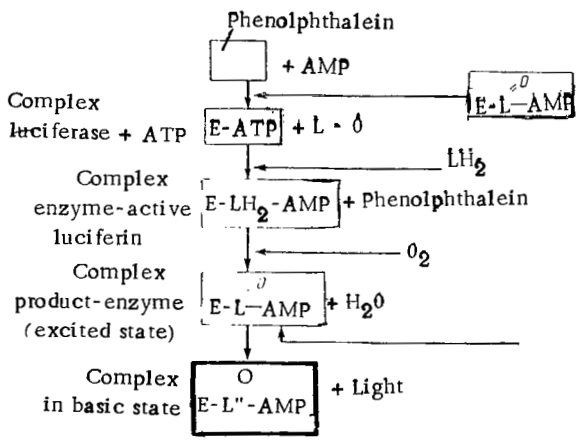
ADENOSINE TRIPHOSPHATE DETERMINATION IN EXTRATERRESTRIAL LIFE DETECTION

S. A. Butenko, L. M. Mukhin, Ye. I. Milekhina

ABSTRACT. Description of a highly sensitive, quantitative ATP-determination method using an extract of the luminescent organs of fireflies. The method is based on the fact that the fermentative oxidation of the luciferin in the extract occurs only in the presence of ATP.

It is well known that adenosine triphosphate (ATP) is a universal agent for energy conversion and transfer in processes of vital activity. In conjunction with the problem of detecting extraterrestrial life, it has become necessary to measure ATP quantitatively. At the present time, a highly sensitive bioluminescent method developed by McElroy and Strehler (1954) is widely used for this purpose. The basis of this method is the fact that in the so-called "firefly" extract obtained from the luminescent organs of fireflies, the enzymatic oxidation of luciferin takes place only in the presence of ATP in the system. McElroy and Seliger (1961) proposed the following series of reactions which in their opinion cause luminescence in the luciferin-luciferase-ATP system in fireflies (see the figure). /54

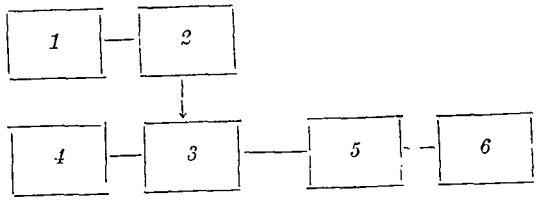


Reactions Proposed by McElroy and Seliger.

The intensity of the glow is linearly dependent on the concentration of ATP. This fact served as the basis for the development of the chemiluminescent method of determining ATP in highly diverse systems: in the cells of microorganisms, in the tissues of animals and plants, etc. However, this method has been used quite rarely in microbiological studies, due primarily to the

extremely small content of ATP in microorganisms and its variation within limits of 10^{-16}-10^{-18} gram of ATP per cell.

Since the minimum amount of ATP that could be determined on Soviet-built instruments in use at the present time is 10^{-9} g/ml (Tumeran and Fedorovich, 1967), quantitative studies have to be performed with highly concentrated suspensions of cells. Hence, it is extremely important to increase the sensitivity of determining ATP. For this purpose, we use the following system which allows measurement of ATP concentrations in solution up to $5 \cdot 10^{-11}$ g/ml (see the drawing).



The stabilized high voltage is supplied by a VS-22 rectifier (1) to the unput of the FEU-42 photomultiplier (2). A quartz reaction cell with a volume of 3 ml is mounted in front of the photocathode of the phototube. /55 The phototube and the cell are mounted in a lightproof housing. The voltage impulses which develop on the anode of the phototube as the result of the bioluminescent reaction are amplified by an impermeable amplifier USh-2 (3) and supplied to the input of a counting-rate integrator ISS-3 (5). The signal is recorded by means of an electronic strip chart recorder with potentiometer, type EPP-09 (6). Visual observation of the reaction process is accomplished with the aid of an electronic oscillograph 40-101 (4). The voltage across the photomulitplier and the amplification mode are selected so that the signal-to-noise ratio is maximum. With the aid of this system, we succeeded in obtaining a reliable signal for values up to 10^{-11} gram of ATP per milliliter of solution.

Method of Preparing Firefly Extract

To determine the amount of ATP from the luminous organs of fireflies, it is necessary to extract the enzyme-substrate complex, luciferase and luciferin. To do this, 166 mg of dried firefly tails were ground in a porcelain mortar

with 50 ml of cold 0.025 M glycyl-glycine buffer (pH 7.8). The suspension was centrifuged for 15 minutes on a K 14 centrifuge (10,000 rpm) at 0°, the precipitate was extracted repeatedly with 50 ml of the same buffer and centrifuged again. The supernatant liquids were decanted together, frozen (then thawed) and stored in a refrigerator for two days. Then the firefly extract was separated from the denatured protein by filtration through filter paper. All operations were carried out at 0 to +4°. The prepared extract was stored in the frozen state. It remained active for six months.

Preparation of Adenosine Triphosphate Solution

One milligram of adenosine triphosphate sodium salt (a preparation obtained from the Hungarian firm of "Reanal") was dissolved in 10 ml of doubly distilled water and by means of a series of dilutions, adenosine triphosphate dilutions from 10^{-1} to 10^{-10} g/ml were obtained.

Preparation of Suspensions of Bacteria and Yeast

In this work, we used two-day cultures of *Escherichia coli*, *Serratia marcescens*, and *Saccharomyces cerevisiae*, grown on tilted meat and must agar, respectively. The cells were rinsed with distilled water and suspensions of microorganisms were prepared according to the turbidity standard (500,000,000 cells). Then the number of cells was determined under the microscope.

Preparation of Soil Extracts /56

The soil samples were placed in a sterile porcelain mortar, a small quantity of distilled water was added, and the soil was carefully stirred for 10 minutes. Then the soil suspension was placed in a magnetic stirrer for 15 minutes and filtered through a No. 6 membrane filter. The soil solution was investigated for its ATP content.

Results of the Studies

Our work involved determining the minimum quantity of ATP required to cause light emission as determined by our instrument. The experiments were performed with a firefly extract prepared with 0.025 M glycyl-glycine buffer (pH 7.8) and 0.1 M tris-buffer (pH 7.4). As we can see from the data listed

below, the luminosity that was observed was more intensive if the reaction took place with an extract prepared with glycyl-glycine buffer.

Concentration of ATP, g/ml	Amplitude of Signal, Relative Units	Concentration of ATP, g/ml	Amplitude of Signal, Relative Units
Glycyl-glycine buffer		Tris-buffer	
10^{-10}	25	10^{-10}	11
10^{-9}	28	10^{-9}	24
10^{-8}	59	10^{-8}	47
10^{-7}	119	10^{-7}	89

A concentration of ATP equal to 10^{-10}-10^{-11} g/ml is the minimum that could be determined with the aid of our ATP-meter.

As far as the cultures of *Escherichia coli*, *Serratia marcescens* and *Saccharomyces cerevisiae* were concerned, the work involves breaking them down by various methods and then determining their ATP content. Aqueous suspensions of these cultures were subjected to heating in a boiling water bath at temperatures up to 100° for 5-30 minutes.

As we can see from the data in Table 1, with the aid of heating ATP may be observed only in the yeast; liberation of ATP takes place only to a very slight degree in the case of *E. coli*. A pure preparation of ATP undergoes insignificant breakdown when heated.

The study of the influence of freezing and thawing on the liberation of ATP from the cells showed that only in the case of *S. cerevisiae* can ATP be determined (10^{-7} g/ml) following repeated freezing and thawing (Table 2).

A study of the effect of ultrasound on the breakdown of cells and the liberation of ATP showed that ATP (10^{-7} g/ml) may be extracted and measured /58
in the case of all the cultures investigated following five minute exposure to ultrasonic disruption (Table 3).

We also performed work involving the study of the effect of lysozyme on the process of liberation of intracellular ATP. The results of the experiments as shown in Table 4 indicate that liberation of ATP can be accomplished under the influence of lysozyme only in the case of *S. cerevisiae*.

/57

TABLE 1. INFLUENCE OF HEATING ON THE PROCESS OF LIBERATION OF INTRACELLULAR ATP

Treatment, minutes	ATP, 10^{-7} g/ml*	*E. Coli*	*Serratia** marcescens*	*Sacch. cerevisiae**
Without heating	98	0	3	5
Heating to 100°				
5	78	10	5	17
10	78	13	0	20
20	78	--	--	--
30	70	7	0	13
60	60	--	--	--

* The numbers indicate the amplitude of the signal on the ATP-meter
**Pure cultures were obtained from the Standard Culture Department of the Institute of Microbiology of the Academy of Sciences of the USSR.

TABLE 2. INFLUENCE OF FREEZING AND THAWING ON THE LIBERATION OF INTRACELLULAR ATP

Culture	Without Freezing	Single freezing and thawing	Triple freezing and thawing
E. coli	12	10	10
Serratia marcescens	3	3	0
Sacch. cerevisiae	7	17	117
ATP, 10^{-7} g/ml	100	100	100

TABLE 3. EFFECT OF ULTRASOUND ON THE DISRUPTION OF CELLS

Culture	Duration of Destruction of Cells by Ultrasound*, minutes			
	0	5	10	15
E. coli	12	102	102	102
Serratia marcescens	9	72	93	93
Sacch. cerevisiae	12	150	135	129

*Destruction took place on the MSE ultrasonic apparatus.

TABLE 4. INFLUENCE OF LYSOZYME ON THE LIBERATION OF INTRACELLULAR ATP

Culture	Duration of Action of Lysozyme*, minutes			
	0	5	30	120
E. Coli	0	3	3	4
Serratia marcescens	3	3	2	2
Sacch. cerevisiae	5	45	43	22

*Concentration of lysozyme equals 1 mg/ml of cell suspension.

We know that lysozyme does not destroy the cell walls of all microorganisms by any means. Therefore, we performed experiments to study the effect of a combination of preliminary freezing and thawing or heating with subsequent destruction of the investigated cultures by lysozyme.

Experiments (Table 5) showed that the cell walls of *Serratia marcescens* and *E. coli* are broken down by lysozyme if the cell suspension is subjected beforehand to a 10 minute exposure to high temperature (100°). *S. cerevisiae* is broken down by lysozyme both following freezing and thawing and following the action of high temperature (Table 5).

TABLE 5. DESTRUCTION OF CELLS BY LYSOZYME WITH PRELIMINARY FREEZING AND THAWING AS WELL AS BY THE ACTION OF HIGH TEMPERATURE IN ORDER TO DETERMINE THE CONTENT OF INTRACELLULAR ATP.

Treatment	*E. Coli*	*Serratia marsescens*	*Sacch. cerevisiae*
Boiling (100°, 10 min.) + lysozyme (30 min.)	78	165	840
Freezing and thawing (3 times) + lysozyme (30 min.)	36	30	1290
Without treatment	12	24	24

TABLE 6. DETERMINATION OF ATP IN SOIL EXTRACT /59

Soil Suspension (soil from Karakum Desert), g/ml	Without Destruction	Method of Destruction		
		Lysozyme, 30°	Boiling, 100°,10 min.	Lysozyme, 30°, boiling for 10 min
0.025	4	10	21	12
0.125	6	26	20	30
0.250	6	27	15	30
0.5	21	12	15	30
1.0	15	6	15	26
ATP, 10^{-9}	30	30	30	30

Tests were performed to determine the amount of ATP in soil extract containing soil microorganisms.

The data listed in Table 6 indicate that it was possible to measure ATP in the soil under investigation following treatment of the soil extract with lysozyme and lysozyme in combination with boiling, freezing and thawing.

Evaluation of the Results

It was discovered in the course of preliminary experiments that the minimum amount of ATP which could be detected with the aid of the luciferin-luciferase system is equal to 10^{-10}-10^{-11} g/ml. Tests were performed with cells of *Saccharomyces cerevisiae*, *Serratia marcescens*, *E. coli*. It was found that boiling, triple freezing and thawing and the action of lysozyme caused liberation of intracellular ATP only in the case of the yeast. For bacteria, these methods yielded completely or partially negative results. However, following preliminary treatment of the bacterial cells by boiling, lysozyme readily broke them down, thereby liberating the intracellular ATP. The destruction of the cells by ultrasound in a period of five minutes also leads to the liberation of ATP. We studied soil from the Karakum Desert, which contains microorganisms in the amount of 10^5 cells per gram. The amount of soil subjected to extraction varied from 0.025 to 1 g/ml.

The soil extracts were subjected to the action of lysozyme, boiling and the combined action of lysozyme and boiling. In untreated soil extracts, ATP could be found. It was in those cases when the extract was prepared from relatively large amounts of soil that the signal amplitude was rather high.

The treatment of the soil extracts with lysozyme or boiling them does /60
not lead to a reliable signal increase, although in some cases a favorable effect is observed. Successive treatment of the soil samples by boiling and with lysozyme leads to the liberation of intracellular ATP and a sharp increase in the signal amplitude.

The examples given, it seems to us, show that the bioluminescent method of ATP determination may find very extensive application under the most diverse biological and biochemical study conditions.

Conclusions

1. ATP may be measured in desert soil extracts with the aid of firefly reaction.

2. Detection of ATP is possible in desert soil which contains 10^5 cells of microorganisms.

3. The quantitative determination of ATP may be used as one of the methods for detection of extraterrestrial life.

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DETECTION OF FERROPORPHYRIN PROTEINS IN EXTRATERRESTRIAL LIFE SEARCHES

G. G. Sotnikov and S. A. Butenko

ABSTRACT. The possibility is studied of using the biochemical luminescence reaction for determining porphyrin containing proteins of microorganisms. It is shown that the kinetics of the reaction with porphyrin-containing proteins differs both in amplitude and in chemoluminescent signal duration from the kinetics of reactions with iron-containing catalysts of nonhemin origin. The kinetics of luminol peroxide reactions with soil microflora, with pure bacterial cultures, and with chemically pure porphyrin-containing protein preparations are altogether identical. No preliminary cell wall dissolution by lysis agents is required for recording the signal of ferroporphyrin presence.

The synthesis of porphyrins is a necessary step in the process of the development of life. Some investigators (Gaffron, 1960; Calvin, 1962) feel that porphyrins played an active role in the earliest stage of formation of organic structures; others (Miller, Urey, 1959) declare that porphyrins may have been formed in the process of evolution of the first organisms. Strughold and Ritter (1962) hold that porphyrins of the heme type arose following /61
the formation of chlorophyll. Thus, Szutka (1963) suggests that in the first stage of chemical evolution, following saturation of the medium by simple organic compounds, conditions arose (ozone screen, absence of UV radiation as an energy source) which made it possible for the porphyrins to appear. Hydrogen peroxide was formed as the reducing conditions that existed in the atmosphere were changed into oxidizing ones. It was necessary to have a mechanism which would provide for rapid breakdown of any peroxide that developed. Calvin (1959) observed that the ability of iron to catalyze the decomposition of peroxide is increased by a factor of 10^3 if the iron atom becomes a part of the porphyrin molecule. However, association of the iron with certain proteins (catalase) increases the rate of decomposition of peroxide by 10^7 times.

Ferroporphyrin proteins, catalase, peroxidase, hemoglobin and cytochrome c are complex enzymes whose prosthetic group is ferroporphyrin IX or its derivatives (Blyumenfel'd, Purmal', 1964). Ferroporphyrin enzymes can be divided into two basic classes: 1) cytochromes, which are oxidizing-reducing enzymes that participate in the transfer of electrons in the respiratory chain; 2) catalase and peroxidase, which are oxidizing-reducing enzymes that used hydrogen peroxide as an acceptor.

The basic function of catalase is to catalyze the reaction of the decomposition of hydrogen peroxide and thereby protect the biochemical systems of the cell from the toxic effect of hydrogen peroxide, which is formed with the participation of flavin oxidizing enzymes.

The total activity of the catalase and peroxidase reactions is referred to as the hydroperoxidase activity (Ponomarenko, 1965). To determine the hydroperoxidase activity, we used a chemiluminescent method based on the oxidation of 3-aminophthalohydrazide (luminol) by hydrogen peroxide in an alkaline medium (Albrecht, 1928; Vasserman, Miklukhin, 1939). The reaction involving the oxidation of the luminol by peroxide has been studied over the last 30 years (Sveshnikov, 1938; Sveshnikov, Dikun, 1945; White, 1961). White studied the oxidation of luminol in aqueous solution. He showed that the luminescence of luminol corresponds to quanta (60-70 kcal/mol). The maximum chemiluminescence is in the 430 nm range, corresponding to 68 kcal/mol.

Any explanation of the mechanism of luminescence of luminol must take into account the fact that both oxygen and hydrogen peroxide are required for chemiluminescence; free radicals and the necessary metal-containing catalysts participate in the reaction. The medium in which the oxidation of the luminol takes place must be alkaline; chemiluminescence is most intensive at a pH of 11 (White, 1961; White et al., 1965).

Experimental Section /62

A system was set up at the Institute of Microbiology of the Academy of Sciences of the USSR to study biochemiluminescence. Figure 1 shows a block diagram of this arrangement.

The reaction cell (1) and the photoelectronic multiplier (2) are enclosed in a metallic lightproof housing (3). The photomultiplier is powered by a high-voltage stabilized rectifier (4). The reaction mixture is added to the cell by means of a plastic syringe with a capacity of 3 ml. At the end of the reaction, the products are drained through the lower opening in the cell. The light signal is transformed in the photoelectronic multiplier into an electrical signal, which is amplified by a wide-band amplifier (5), integrated (7) and recorded by an automatic potentiometer (8). The pattern of the luminescence can only be observed visually by means of an oscillograph (6). The voltage supplied to the FEU-42 is 600 volts. The amplification is 30,000. The range of measurement on the pulse counting rate integrator ISS is equal to $5\text{-}15 \cdot 10^3$ impulses/sec. The background from the luminol-H_2O_2 is $5 \cdot 10^1$ impulses/sec.

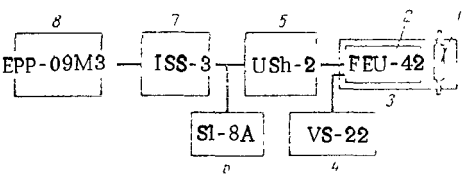

Figure 1. Diagram of Apparatus For Biochemiluminescence.

Preparation of Reaction Mixture

The reaction mixture (luminol + peroxide) is prepared by dissolving 50 mg of luminol in 100 ml of 0.1 N NaOH, after which 0.2 ml of a 30% solution of H_2O_2 (Voronov, Kononenko, 1966) is added to the solution. 2 ml of the reaction mixture is then added by means of a plastic syringe to a cell with a capacity of 3 ml, after which the same syringe is used to add 1 ml of the catalyst being studied to the cell or else a suspension of microorganisms. The use of a metal syringe is not recommended. The cell is rinsed out with distilled water.

Kinetics of the Luminol-Peroxide Reaction With Various Components

During the oxidation of luminol by peroxide, there is a prolonged chemiluminescence of the luminol which is constant in intensity. Against the background of this luminescence, when luminol-peroxide ions of iron are added to the system in the form of a solution of $FeSO_4$ salts or iron citrate, there is an increase in the signal intensity in the form of a peak on the curve and /64

a rapid drop in the course of several seconds (Figure 2). The kinetics of this reaction following the introduction of a biological catalyst consisting of either pure preparations of prophyrin-containing protein (Figure 3) or microorganisms and spores containing these proteins (Figure 4) differs markedly in intensity and quite strongly during the course of the decline in luminescence.

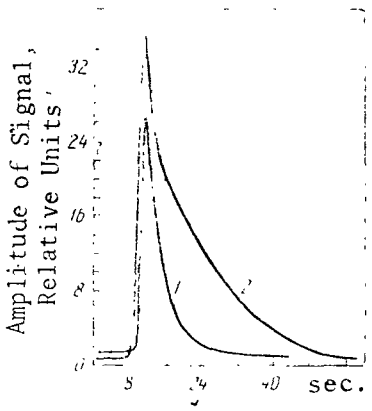

Figure 2. Kinetics of the Reaction Involving Luminol + H_2O_2 With Catalysts of Nonhemin Origin.

1, $FeSO_4$-concentration of 10^{-4} M; 2, iron ammonium citrate in a concentration of $1.2 \cdot 10^{-3}$ g/l in a liquid nutrient medium for hydrogenous bacteria.

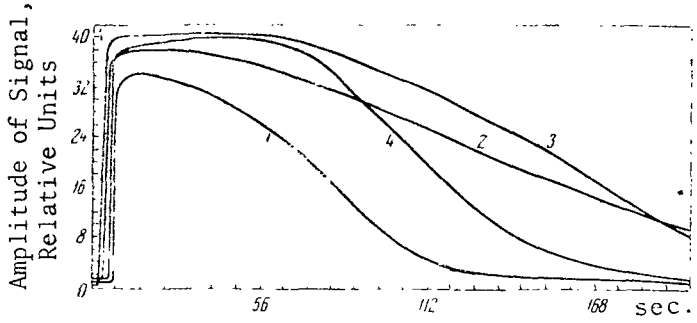

Figure 3. Kinetics of the Luminol + H_2O_2 Reaction With Catalysts of Hemin Origin.

1, catalase, concentration equals 10^{-6} M;
2, peroxidase, concentration equals 10^{-7} M;
3, cytochrome c, concentration equals 10^{-8} M;
4, hemoglobin, concentration equals 10^{-9} M.

Obtaining Bacterial Protoplasts

We studied the effect of various lytic preparations on the cell walls of *E. coli* and *B. megaterium* for the purpose of extracting the porphyrin-containing proteins and obtaining the maximum biochemiluminescence signal. The following preparations were investigated: Japanese lysozyme (firm of "Nagase"), Japanese celluzyme (firm of "Nagase"), Hungarian lysozyme (firm of "Reanal") and preparations of cellulolytic enzymes prepared from filtrates of culture liquids from *Aspergillus terreus* and *A. fumigatus* (Loginova, Tashpulatov, 1967). The lytic substances were added in various concentrations to the suspensions of bacterial cells. The density of the suspension was $5 \cdot 10^8$ cells per ml. The cells were counted in a Goryayev

chamber. Then the cells were incubated at 28 and 40° for several hours in a medium with the following compositions: glucose -- 10 grams, NH_4NO_3 -- 0.5 gram, asparagine -- 0.1 gram, KH_2PO_4 -- 0.91 gram, K_2HPO_4 -- 2.61 gram $MgSO \cdot 7H_2O$ -- 0.3 gram, $CaCl_2$ -- 0.1 gram, NaCl -- 0.1 gram, solution of a mixture of microelements -- 1 ml, yeast autolysate -- 5 ml, liver extract -- 0.1 ml, distilled water -- 1 liter.

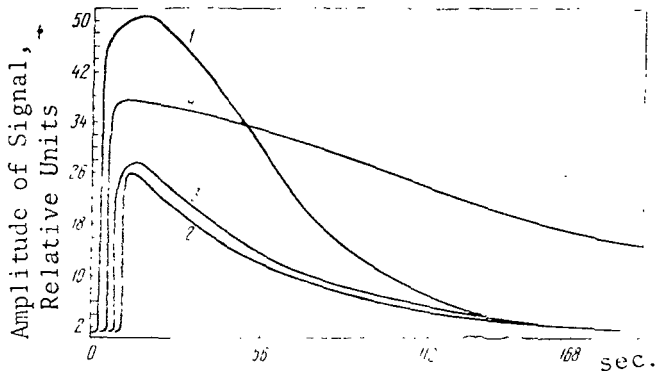

Figure 4. Kinetics of the Luminol-Hydrogen Peroxide Reaction With Pure Cultures of Nonsporiferous and Sporiferous Bacteria.

1, Hydrogenous bacteria *Hydrogenomonas* Z-1, cell concentration in the nutrient medium equals $1 \cdot 10^9$ cells per ml; 2, *E. coli*, concentration of $1.5 \cdot 10^8$ cells per ml; 3, *B. megaterium*, concentration of $4 \cdot 10^7$ cells per ml; 4, spores of *B. simplex*, concentration of $1 \cdot 10^9$ cells per ml.

To suppress synthesis and increase the permeability of the cell wall, penicillin was added in the amount of 1,000 units per ml of cell suspension (Martin, 1963). As a control, we used bacterial cells without addition of a lytic preparation and penicillin, as well as cells with penicillin alone.

Photometric Determination of the Effect of Lytic Preparations on Cells of *E. coli* and *B. megaterium*

The effect of lytic preparations on microorganisms was examined with the aid of the SF-4A spectrophotometer, since the suspension became more transparent as the cells were lysed. The photocell was replaced by a more sensitive light sensor -- the photoelectronic multiplier, FEU-42. The recording

of the signal was accomplished with an electronic strip chart recording device for measuring and recording small current (EPPV-60 MZ). Measurements of the optical density were performed at a wavelength of 480 nm. The optical /66
density of the corresponding culture of cells in the nutrient medium at the beginning of the experiment was used as the starting point for the measurements.

Results of the Studies

In determining the lytic action of various concentrations of celluzyme and preparations of cellulolytic enzymes from the Aspergillus fungi, it was found that these preparations have a slight inhibiting effect on the multiplication of cells of *E. coli* (Figure 5, a-d) at both 40° and 28°, regardless of the time of action and have a slightly greater inhibiting effect on the multiplication of cells of *B. megaterium* (Figure 5 e-h). The maximum lytic effect of cells on *E. coli* and *B. megaterium* was shown by Japanese lysozyme with addition of penicillin. It should be pointed out that in the range of concentrations from 0.1% lysozyme to 0.01%, the lytic effect increases with /67
a decrease in the concentration of preparation. Concentrations of lysozyme equal to 0.0025, 0.005 and 0.01% have about the same lytic effect on the cells (Figure 6, a-f). From the data given above, it is clear that the most suitable preparation for breaking down the bacteria must be considered to be Japanese lysozyme. Therefore, for our further work in determining the porphyrin-containing proteins, we used Japanese lysozyme in a concentration of 0.01%. The dependence of the signal amplitude for the porphyrin-containing proteins on the cell concentration (breakdown with the aid of Japanese lysozyme, 0.01%, 2 hours at 37°) is shown below. Initially we determined the limiting sensitivity of the apparatus for chemiluminescence equal to 10^5-10^6 cells per ml.

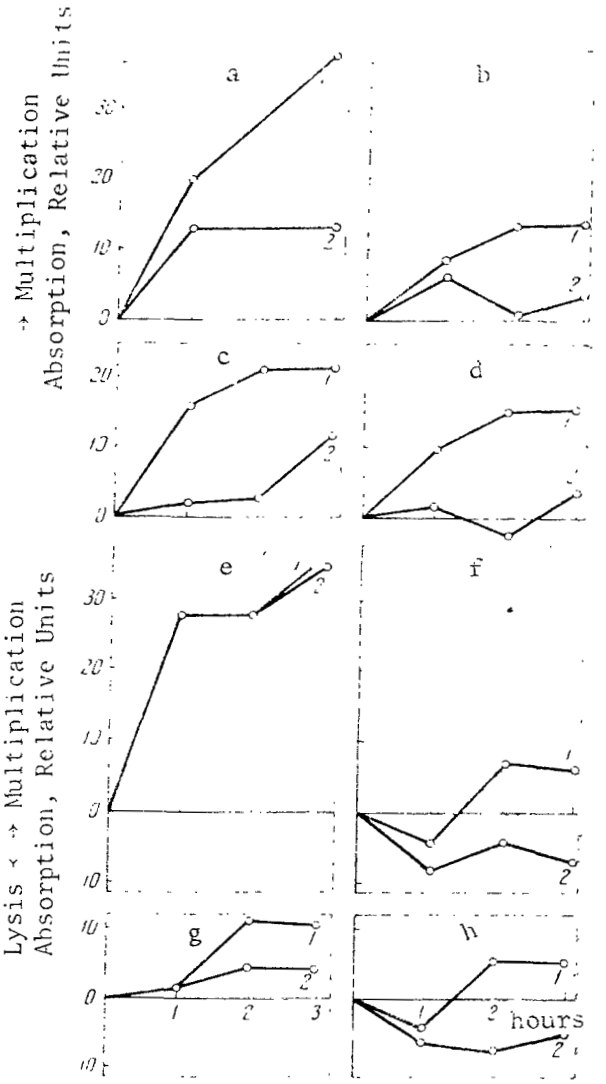

Figure 5. Photometric Determination of Lytic Effect of Celluzyme and Preparations of Cellulolytic Enzymes Obtained from Cultures of *Aspergillus terreus* and *A. fumigatus* on cells of *E. coli* (a-d) and *B. megaterium* (e-h); Incubation temperature equals 40°. Relative absorption was measured at a wavelength of 480 nm.

a, e: 1, Control; 2, Penicillin; b, f: 1, filtrate from *A. terreus;* 2, filtrate + penicillin; c, g: 1, celluzyme; 2, celluzyme + penicillin; d, h: 1, filtrate from *A. fumigatus*, 2, penicillin.

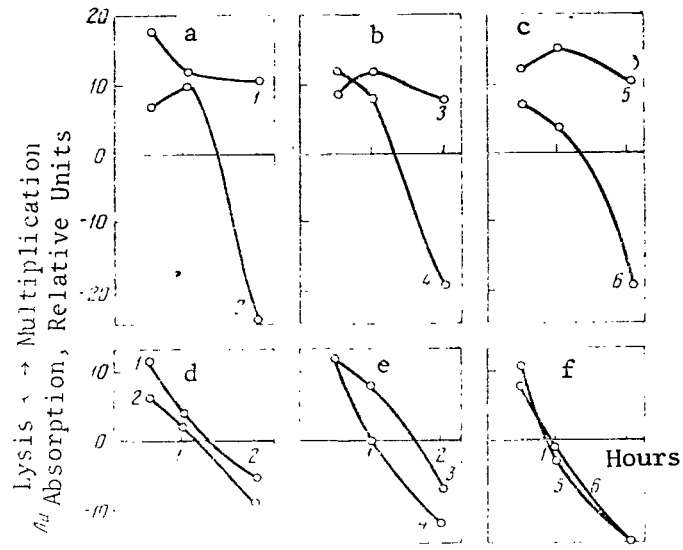

Figure 6. Photometric Determination of the Effect of Lysozyme on Cells of *E. coli* (a-c), and *B. megaterium* (d-f).

1, Lysozyme in a concentration of 0.01%; 2, lysozyme (0.01%) + penicillin; 3, lysozyme (0.005%; 4, lysozyme (0.005%) + penicillin; 5, lysozyme (0.0025%); 6, lysozyme (0.0025%) + penicillin.

No. of Cells per ml of nutrient medium	Amplitude of signal, relative units	No. of cells per ml of nutrient medium	Amplitude of signal, relative Units
E. coli		*Bac. megaterium*	
$1.5 \cdot 10^8$	28	$4 \cdot 10^7$	23
$3 \cdot 10^7$	20	$8 \cdot 10^6$	18
$6 \cdot 10^6$	12	$1.6 \cdot 10^6$	12
$1.2 \cdot 10^6$	2	$3 \cdot 10^5$	2

It should be pointed out that the magnitude of the signal for porphyrin-containing proteins is of the same order both for cells treated with Japanese lysozyme and those with addition of the latter. The data for *E. coli* are presented below.

Preparation	2 hours	3 hours
Suspension of *E. coli* cells ($1.5 \cdot 10^8$ cells per ml, prepared in distilled water)		
Japanese lysozyme, 0.01%	38	41
Japanese lysozyme, 0.01% + penicillin, 1,000 units/ml	34	36
Penicillin, 1,000 units/ml	31	32
Control (without destruction)	28	29
Suspension of *E. coli* cells ($1.5 \cdot 10^8$ cells per ml, prepared in physiological solution)		
Japanese lysozyme, 0.01%	27	30
Japanese lysozyme, 0.01% + penicillin, 1,000 units/ml	26	30
Penicillin, 1,000 units/ml	27	28
Control (without destruction)	27	29

The intensity of the chemiluminescent signal from the cells treated with ultrasound for five minutes differs by 3-4 relative units from the signal of the intact cells.

We then performed experiments involving detection of porphyrin-containing proteins in microorganisms in soil that was poor in organic substances. For /68
this purpose, we used the sandy soil from the Karakum Desert (Repeteka rayon). The data of Sagan and Pollack (1966) indicate that on the surface of Mars there is a predominance of silicates with a high content of oxidized iron (limonite); this is also characteristic of this soil. Comparative data for *B. megaterium* are presented below; in both the first and second cases, the numbers correspond to the magnitude of the signal amplitude in relative units for porphyrin-containing proteins.

Preparation	2 hours	3 hours
Suspension of cells of *B. megaterium* ($4 \cdot 10^7$ cells per ml, prepared with distilled water)		
Japanese lysozyme, 0.01%	13	20
Japanese lysozyme, 0.01% + penicillin, 1,000 units/ml	24	28
Penicillin, 1,000 units/ml	24	24
Control (without destruction)	9	23
Suspension of cells of *B. megaterium* ($4 \cdot 10^7$ cells per ml, prepared with physiological solution)		
Japanese lysozyme, 0.01%	14	23

Preparation	2 hours	3 hours
Japanese lysozyme, 0.01% + penicillin, 1,000 units/ml	26	23
Penicillin, 1,000 units/ml	28	28
Control (without destruction)	11	20

200 mg of soil were added to 100 ml of the above-described medium. The culture temperature was 37°. Since iron ions catalyze the oxidation reaction of liminol by peroxide, it was necessary to clarify the effect of limonite ($Fe_2O_3 \cdot nH_2O$) on the intensity and kinetics of the chemiluminescence signal. For this purpose, 100 mg of sterile limonite was added to 100 mg of sandy soil. As a control, we used a flask with sterile desert soil (200 mg per 100 ml of medium) and sterile limonite (200 mg per 100 ml of medium). After 4, 7, 10, 13, 22 and 48 hours of incubation, samples of culture fluid were selected and examined by a biochemiluminescent method with preliminary treatment for two hours with a solution of (0.01%) of Japanese and Hungarian lysozyme. In some of the experiments, the cells were destroyed in five minutes by ultrasound. The experiments showed that after only 13 hours following inoculation it was possible to obtain a reliable signal indicating the presence of porphyrin-containing proteins belonging to the microflora of the desert soil. The method of destroying the cells was not reflected in the results (Figures 7 and 8). It is clear from the figures that the background from the sterile sand and sterile limonite was insignificant -- 2-4 relative units (curves 5-8).

It should be pointed out that the kinetics of the luminol-hydrogen peroxide reaction with limonite is similar to the reaction for which ionic iron is the catalyst (Figure 9, 6). The kinetics of the luminol-hydrogen peroxide reaction with soil microorganisms (Figure 9) is identical to the kinetics with pure cultures of bacteria and with chemically pure preparations of porphyrin-containing proteins. While the signal amplitude from the intact and destroyed (by ultrasound) cells is the same, the decrease in the intensity of the luminescence is completely different (Figures 9, 3, 4). The total light flux during the period of time which elapses from the maximum of the luminescence to its minimum for cells destroyed by ultrasound is almost twice as great as for intact ones.

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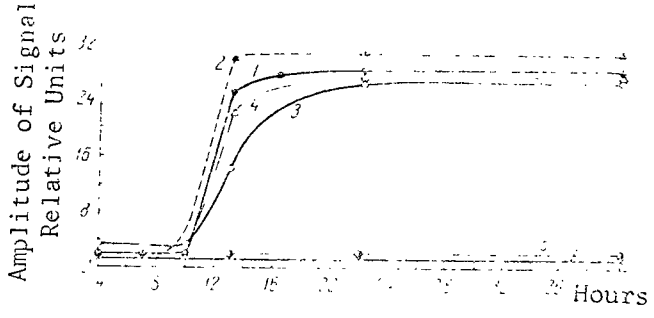

Figure 7. Dynamics of the Change in the Biochemiluminescent signal Following Seeding of Nutrient Medium with Desert Soil (Karakum, Repetek).

1, Without destruction; 2, destruction by ultrasound, 5 minutes; 3, without destruction (desert soil + limonite); 4, destruction by ultrasound, 5 minutes (desert soil + limonite); 5, limonite, sterile; 6, sterile desert soil.

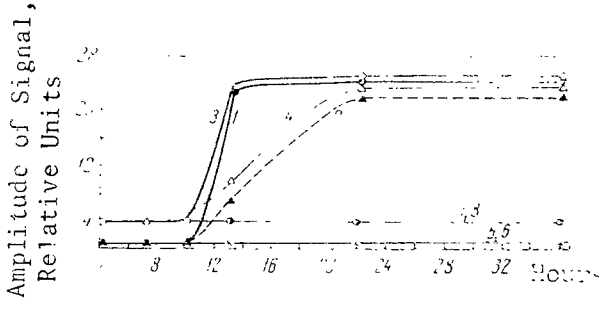

Figure 8. Dynamics of the Change in Bioluminescent Signal Following Seeding of Nutrient Medium With Desert Soil (Karakum, Repetek).

1, Destruction by Japanese lysozyme -- 0.01% (desert soil); 2, destruction by Japanese lysozyme -- 0.01% (desert soil + limonite); 3, destruction by Hungarian lysozyme -- 0.01% (desert soil); 4, destruction by Hungarian lysozyme -- 0.01% (desert soil + limonite); 5, sterile desert soil, destruction by Japanese lysozyme; 6, sterile limonite, destruction by Japanese lysozyme; 7, sterile desert soil, destruction by Hungarian lysozyme; 8, sterile limonite, destruction by Hungarian lysozyme.

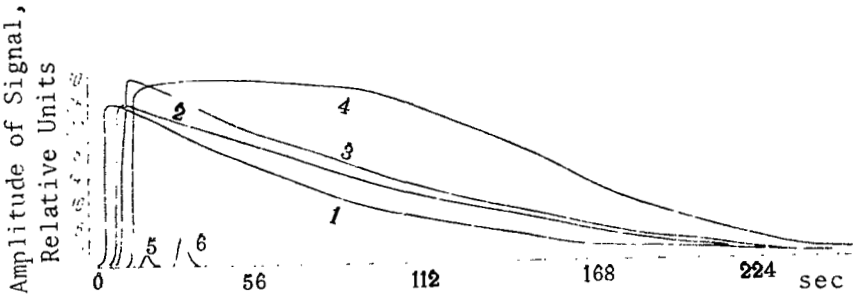

Figure 9. Kinetics of Luminol-Hydrogen Peroxide Reaction As A Function of Methods of Processing Microflora from Desert Soil.

1, Without destruction, incubation time 13 hours at 37°; 2, destruction by Hungarian lysozyme, incubation time 13 hours at 37°; 3, without destruction, incubation time 22 hours at 37°; 4, destruction by ultrasound in 5 minutes, incubation time 22 hours; 5, signal from Hungarian lysozyme; 6, Signal from sterile limonite, $Fe_2O_3 \cdot nH_2O$.

Conclusions

1. We studied the possibility of using biochemiluminescent reaction of luminol-hydrogen peroxide to determine the porphyrin-containing proteins in microorganisms.

2. It was shown that the kinetics of the reaction involving porphyrin-containing proteins differs both in terms of the amplitude of the signal and in the duration of the decline in luminescence from the kinetics of the reaction with catalysts of nonhemin origin.

3. Preliminary destruction of the cell walls by lysing preparations is not a necessary condition.

4. The presence of iron-porphyrin proteins in the bacteria may be established in the culture fluid after 13 hours following seeding of the desert soil.

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EVALUATION OF LABELLED CARBON DIOXIDE AS ONE METHOD OF DETECTING EXTRATERRESTRIAL LIFE

R. I. Fedorova and L. M. Mukhin

ABSTRACT: A method is described based on the study of the dynamics of separation of labeled carbon dioxide from a substrate when various types of soil are cultured on media containing radioactive glucose as the source of carbon. The proposed method makes it possible to determine the activity directly in a reaction cell and record the results on an electronic strip chart potentiometer. It is suitable for instances when labeled substances in the substrate may change to the gas phase and vice versa in the course of vital activity of organisms. A diagram is provided of the cells and the apparatus which makes it possible to perform recordings on several channels simultaneously.

Labelled atoms are widely employed in biology for studying processes of vital activity in organisms. Modern apparatus makes it possible to determine with a high level of accuracy the quantity of radioactive substance which participates in metabolic reactions. It should be mentioned, however, that the study of the dynamics of any process using the labelled atom method is very laborious and requires a great many operations involving the selection of samples and the measuring of the activity.

In this connection, we are describing in this paper a method for continuous reading and a design for a system which will make it possible to perform measurements in several reaction vessels with recording of the results on an electronic automatic recording potentiometer.

The proposed method is suitable for cases when the labelled substances of a substrate may change to the gaseous phase in the process of the vital activity of the organisms and vice versa (study of fermentation processes, photosynthesis, respiration, etc.). We have used it to study the dynamics of the excretion of carbon dioxide from the substrate during seeding with various types of soil on media containing radioactive glucose as the carbon dioxide source. This problem arose in conjunction with the devising of methods of detecting signs of life on Mars. This property was first suggested by

Levin et al., (1964) for detecting extraterrestrial life by means of the "Gulliver" apparatus.

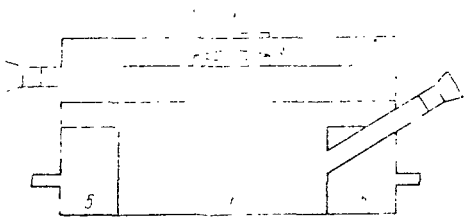

Figure 1. Schematic Apparatus For The Cell.
For the explanation, see the text.

A schematic diagram of the cell for performing the experiments is shown in Figure 1. /72

The lower thermostatically controlled portion of the cell (1) is intended for the culturing of microorganisms. A given temperature is maintained in the cell by means of an ultrathermostat (5). For absorbing radioactive carbon dioxide from the gas phase, a glass cup-target (3) with a slit is located immediately beneath the Geiger counter (4). The counter is protected against the radiation from the labelled substrate by two glass disks (2) which overlap one another by virtue of their parallel mounting and are located at different heights. In this system, it is possible to use isotopes that do not have too strong a radiation spectrum (soft gamma- and beta-radiation), since the protective glass disks have a thickness of about 2 mm. To ensure uniform penetration of the carbon dioxide which is formed from the entire culture medium into the gas phase, the medium is continuously agitated. For this purpose, the cell is mounted on a magnetic stirrer. There are two openings in the cell to add media and inoculate and to fill the chamber with a gas of the required composition. During the experiment, both openings are sealed hermetically. A gas-tight connection between the cell and the Geiger counter is provided by a rubber gasket and a removable cover.

A block diagram of the arrangement is shown in Figure 2. High voltage from a high-voltage block in the "Volna" apparatus (1) is supplied simultaneously to six type SBT counters (2) connected to the reaction cell. The impulses from the counters pass to the input of the six-channel switching /73

device (3) which can pass the impulses to any channel.[1] The channels are switched manually, but a connection to the commutator of a programed device is provided in the circuit. From the output of the commutator, the impulses pass to a PS-10,000 counting circuit (4) and to a counting rate meter (ISS-3) (5), whose output is connected to an electronic potentiometer (type EPP-09) (6). Since the time (τ) for counting on the six channels is much shorter than the time T in which a significant change in the activity takes place ($\tau \ll T$), there is no "loss" of information in the experiment. In this circuit, the number of channels was selected arbitrarily, and this parameter of the device is not critical. The moment that the channels are switched, a mark is left on the strip-chart recorder tape. The appearance of a signal which exceeds the background and its increase with time correspond to the appearance of radioactive carbon dioxide in the cell and its accumulation.

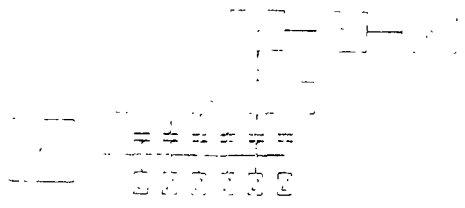

Figure 2. Block Diagram of the Apparatus. Explanation in the text,

In our work, we used a nutrient medium with the following composition (in g/l): glucose, 1-6-C^{14}; NH_4NO_3 -- 0.5; asparagine -- 0.1; KH_2PO_4 -- 0.91; K_2HPO_4 -- 2.61; $MgSO_4 \cdot 7H_2O$ -- 0.3; $CaCl_2$ -- 0.1; NaCl -- 0.1; yeast autolysate -- 5 ml; liver extract -- 0.1 ml; mixture of microelements according to Fedorov and Muromets -- 1 ml; distilled water -- 1 liter. Temperature of cultivation -- 37°.

To create anaerobic conditions, nitrogen was blown through the cell before the start of the experiment. As a control, we used a cell with the same amount of medium and inoculate, but with addition of inhibitors that suppress the vital activity of microorganisms.

We studied the following samples of soil: 1) krasnozem; 2) desert soil (Karakum, Darvaza); 3) tundra soil (matrix-diabase).

[1] The commutator was designed at the A. Ye. Voronkov Physics Institute of the Academy of Sciences of the USSR.

As indicated by experiments with krasnozem, the concentration of labelled glucose in the substrate has no effect within known limits on the nature of the curve showing liberation of carbon dioxide with time (Figure 3). In the case of inoculation of the culture medium with 1 gram of soil, a significant increase in the count was observed approximately 3-3.5 hours following the start of the experiment, regardless of what quantities (20, 40 or 80 microcuries) of labelled glucose were contained in the original medium. The increase in the concentration of labelled glucose in the medium to 200 microcuries, corresponding to 0.5% content of sugar in the medium, increased the count level, but still did not change the nature of the curve for the increase in $C^{14}O_2$ (Figure 4).

Figure 5 shows the curve representing the increase in the count in impulses per 100 seconds following seeding with desert soil from Darvaza. The curves for the increase in $C^{14}O_2$ in experiments with soil from the tundra are /75
completely different. Both in the case of inoculation with nutrient medium with 1 gram (Figure 6) and with introduction of 50 mg of soil to the cell (Figure 7), the device recorded the appearance of labelled carbon dioxide immediately following the start of the experiment.

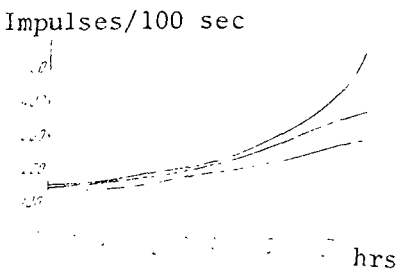

Figure 3. Liberation of Labelled Carbon Dioxide Following Inoculation with 1 gram of Krasnozem on a Medium with a Different Original Content of Radioactive Glucose.
1, 20 microcuries; 2, 40 microcuries; 3, 80 microcuries.

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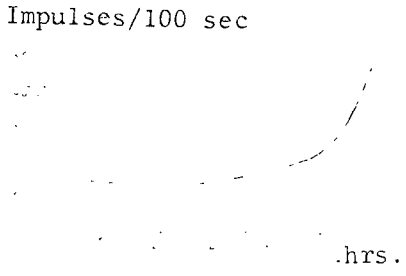

Figure 4. Liberation of Labelled Carbon Dioxide With Seeding of 1 gram of Krasnozem on a Medium With Radioactive Glucose, (200 microcuries).

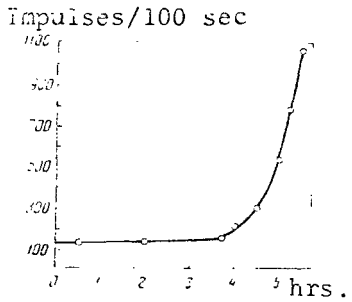

Figure 5. Liberation of Labelled Carbon Dioxide With Seeding of Desert Soil on a Medium with Radioactive Glucose (40 microcuries).

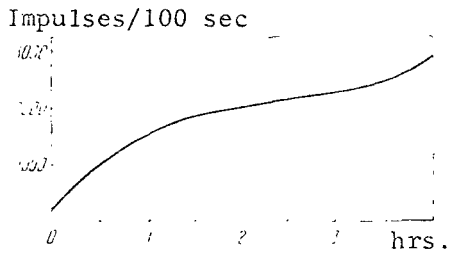

Figure 6. Liberation of Labelled Carbon Dioxide With Seeding of 1 gram of Soil from the Tundra on a Medium Containing Radioactive Glucose (20 microcuries).

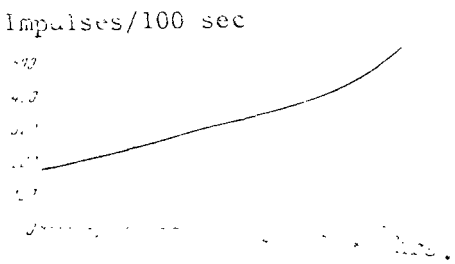

Figure 7. Liberation of Labelled Carbon Dioxide With Seeding of 15 mg of Soil from the Tundra on a Medium With Radioactive Glucose (40 microcuries).

Parallel determination of the quantity of microorganisms in the medium with the above mentioned composition by means of the dilution method revealed that 1 gram of desert soil contained $6 \cdot 10^3$ microbes, while 1 gram of soil from the tundra contained a quantity three orders of magnitude larger -- $6 \cdot 10^6$.

It is obvious that when a signal appears indicating the beginning of liberation of active carbon dioxide from the labelled substrate, the nature of the curve representing the growth and activity is affected not only by the quantity of soil collected for analysis but also by the qualitative composition of the microflora (the ratio of the amounts of aerobic and anaerobic organisms, predominance of vegetative or spore forms, etc.).

Hence, the method of indication of life, based on the use of nutrient media containing labelled compounds, provides clear and reliable results. It is also valuable because when a sufficient quantity of seeding material is introduced the presence of viable microorganisms can be determined 1-3 hours

following seeding. Moreover, the nature of the change in the frequency of the impulses with time can provide some idea regarding the composition of the microflora in the sample under study.

Conclusions

1. Seeding of soil in a nutrient medium with labelled glucose leads to the consumption of the glucose and the liberation of labelled carbon dioxide.

2. Following seeding of the soil from the desert, liberation of labelled carbon dioxide takes place less intensively than when soil from the tundra is used for seeding.

3. As the quantity of labelled glucose added to the nutrient medium is increased, the level of activity increases as well.

4. The determination of the amount of labelled carbon dioxide may be used both for high speed methods of detecting extraterrestrial life (2-3 hours) as well as for longer (5-6 hours) periods of multiplication of micro-organisms contained in the soil under study.

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DEVICE FOR DETECTING EXTRATERRESTRIAL LIFE

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G. A. Kazakov

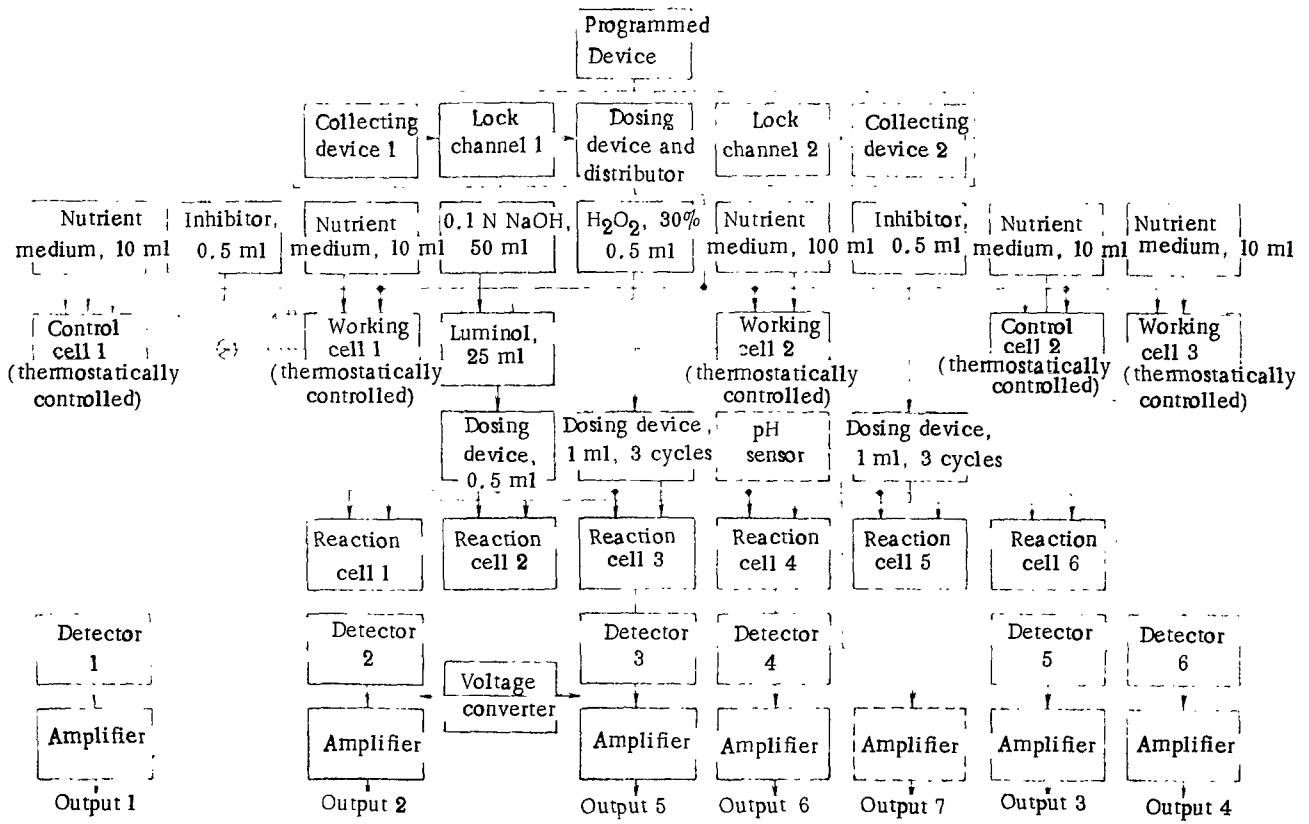
ABSTRACT: A structural diagram is provided for an apparatus for detecting extraterrestrial life, the basis of whose operation is three of the most characteristic methods of measuring the dynamics of multiplication of microorganisms -- the photometric method, the radiometric method and the bioluminescent method, based on measurement of a chemo-luminescent reaction. The paper lists the operations which the apparatus performs. The sequence of the stages in the operation of the apparatus is discussed and the design of several elements of the apparatus is given.

Automatic biological stations which are sent to other planets must be low in weight, reliable and sensitive. In order to obtain the most reliable data on the existence of extraterrestrial life, the device must have several channels and employ several methods of measuring the dynamics of multiplication of microorganisms.

Many devices have been designed for use in the search for extraterrestrial life (the "Gulliver" multivator, the Wolf trap, "Diogenes", the NASA bio-laboratory), based on various physical and physico-chemical methods employed in the biological and biochemical studies of microbiological cultures, protein structures, enzymes, etc. (Levin, Allen, 1965; Buckendahl et al., 1965; Neuman, 1965a, b).

On the basis of theoretical assumptions and experimental studies performed at the Institute of Microbiology of the Academy of Sciences of the USSR (Imshenetskiy et al., 1967), a structural design was worked out for a device for detecting extraterrestrial life. A block diagram of the apparatus is shown on page 91.

Three of the most characteristic methods of measurement are employed as the operating basis of the device.



Structural Diagram of a Device For Detecting Life on Other Planets

I. The photometric method, based on the measurement of variations in the optical density of a medium, produced by the accumulation of cellular biomass and products of vital activity of microorganisms in the process of contact between a soil sample and a nutrient medium under thermostatically controlled conditions during a specific time interval.

II. The radiometric method, based on measurement in the gas phase of radioactive carbon dioxide $C^{14}O_2$, formed as the result of the breakdown of C^{14}-labelled glucose in the nutrient medium in the course of the vital activity of microorganisms.

III. The bioluminescent method, based on a measurement of the chemoluminescent reaction in alkaline solutions of luminol in the presence of hydrogen peroxide under the catalyzing action of enzymes from microorganisms containing ferroporphyrin compounds.

Since the first stage of the investigation of a planet by means of automatic landing devices will begin with a complex study of physical conditions existing on the planet, it is natural that at this stage the device for detecting signs of life on the planet must be installed as the component of the /78
automatic station and must carry out purely analytical functions.

The design of the apparatus allows the following principal operations to be carried out.

1. Sterile collection of soil from the surface of the planet at a distance of several meters from the automatic station.

2. Sterile mixing of measured doses of soil with nutrient media prepared on Earth and subsequent storage of these mixtures for a certain time interval established by the programmed devices.

3. Periodic measurement of the dynamics of multiplication of microorganisms.

All of the operations described above can be carried out either by a previously set rigid program for a programmed device mounted in the apparatus itself or according to a program set by the automatic station.

In addition, the automatic station must provide the following conditions for normal operation of the device: 1) a given stabilized position of the apparatus relative to the surface of the planet; 2) monitoring and maintenance of the temperature and pressure in the device at a specific level; 3) supplying the device with electric power (+ 27 volts d.c.) in required amounts; 4) collection of information from the outputs of the measurement channels of the device at a specific time, conversion and transmission of information to telemetry channels or to a memory block for subsequent transmission to Earth; 5) checking the operation of the individual components of the device.

The design of the apparatus takes the form of an independent block which is firmly linked to the basic design of the automatic station.

The device has two identical lock channels for collecting samples of soil from the planet, mounted at diametrically opposite poles. This design for the collecting device increases the reliability of performance of operations involving collection of soil samples in the event that one of the channels breaks down or in the event that some object is located in front of the opening of the collecting channel.

Sample collection is performed by means of a sticky tape which is extended by an ejection device and drawn back into the apparatus by means of an electric drive.

One gram of the collected soil is collected by the device for measurement and distribution among the working cells. As the strip is drawn back into the device, it passes between two disks which remove particles of soil; under the influence of their own weight, they fall on the intake disk of the dosing mechanism, balanced by a 25 mg counterweight. As soon as the weight of the soil that has fallen on the disk reaches 25 mg, the disk of the dosing device /79
rotates on a hinge and drops the soil into a working thermostatically controlled cell located beneath the disk. At the same time, the disk closes the contacts of a dose counting relay, turns on a stepping motor to move the tilting block of cells, and the next cell moves into position beneath the dosing device.

In the event of nonoperation of the dosing device, an electromagnet moves the dosing device away from the intake opening of the cell and the addition of soil to the cell is measured in terms of time.

The dose selected for the photometric channel is 25 mg, that for the bioluminescent channel is 500 mg and that for the radiometric channel is 100 mg.

After the soil has been distributed among the working cells and the strip has been drawn back into the lock device, a mechanism operates to seal the lock opening. A pressure regulator raises the pressure in the device to a set value. The stepping device moves the block of cells to the working position which corresponds to strict coincidence of the intake and outlet openings of the working cells. Then the mechanism for sealing of the cells operates.

On command from the programmed device, the powder charges on the vessels (ampoules with nutrient medium and inhibitors) operate. These solutions enter the working and control cells, coming into contact with the soil previously introduced. The mechanism for thermostatic control of the working and control cells is switched on. The process of incubating the microorganisms begins.

After the electronic blocks of the photometric and radiometric channels are switched on, information can be collected from the corresponding outputs (outputs 1-4).

To perform measurements by the bioluminescent method, six reaction cells with identical operation are included in the device. Measurement is performed in the following sequence: 1) 1.5-2 hours prior to the start of the first measurement, a mixture of an alkaline solution of luminol with hydrogen peroxide is prepared from components stored in individual hermetically sealed ampoules; 2) the mixture of reagents is poured into two reaction cells -- the first and the fourth in that order; 3) the background from the mixture of reagents is measured; 4) the culture fluid is admitted through dosing devices from the working cell; 5) the useful signal in the first and fourth cells is measured; 6) the tilting mechanism moves the block of reaction cells to the

angle at which two reaction cells (the first and the fifth) are in a position for measurement. Then the apparatus is ready for the next measurement cycle which begins with addition of the reaction mixture.

The device is designed for three measurement cycles. Each measurement is performed with two repetitions. The time between measurement cycles is established by a programmed device.

Vibration-resistant photomultipliers are used as radiation detectors for /80
the photometric and bioluminescent channels, while beta-counters are used for the radiometric channels. Interchangeable filters are provided in the optical block of the photometric channel; these filters allow measurement of optical density of the medium in various regions of the spectrum. The apparatus has a voltage converter for supplying the radiation detectors.

The volumes of the doses shown in the diagram may be reduced by a factor of two. This reduces somewhat the accuracy of the measurement, but makes it possible to decrease the size and weight of the apparatus. Precise dose volumes will be established following laboratory tests for the apparatus.

This design for the mechanism is the result of generalization of experimental studies performed at the Institute of Microbiology of the Academy of Sciences of the USSR.

The device makes it possible to collect data for the planning and construction of new systems for biological study of the planets.

All of this considerably facilitates the solution of problems which arise and will arise in designing large biological laboratories that will include facilities for carrying out a great many physical, physico-chemical and biological studies.

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RESISTANCE OF *COLPODA MAUPASI* INFUSORIA TO LOW PRESSURE, ANOXIA, AND INTENSE COOLING

V. N. Bychenkova and L. K. Lozina-Lozinskiy

ABSTRACT. The resistance and adaptation of the simplest organisms to extremal influences are investigated, and their tolerance of conditions similar to those on Mars is studied. Results of experiments, using a special apparatus imitating some Martian life conditions, indicate that *Colpoda* infusoria are capable of reproduction in a flow-through atmosphere of air or nitrogen containing mere traces of oxygen. At rest in latency, they are resistant to intense cooling, as well as to ultraviolet irradiation.

In exobiological studies, bacteria, unicellular and multicellular plant organisms serve almost exclusively as the objects. It would be equally interesting to study the possibility of existence of representatives of the animal kingdom under extremal conditions similar to those existing on Mars. Model objects for experiments in this connection could be certain very simple animals, in particular those infusoria which do not require considerable amounts of oxygen, form rest-stage cysts and are capable of withstanding various environmental factors which are unfavorable for life. Among the most important factors that limit the active existence of animal organisms under Martian conditions are the following: negligible content of oxygen in the atmosphere, low pressure, temperatures with high diurnal variations, low content of moisture and possibly shortwave ultraviolet radiation.

We studied the reactions of our chosen objects, the infusorian *Colpoda maupasi* in terms of its reaction to UV-radiation in the short wave region (λ = 2537 A) and the diurnal variations of temperature (Bychenkova, 1966). This article presents data on the reaction of this infusorian to low pressure in an atmosphere of air and nitrogen and severe cooling at low pressure.

It is assumed that the atmospheric pressure of Mars is 10 to 12 times less than that on Earth and roughly equal to the pressure which is found in

the stratosphere at an altitude of 17 km (83-87 mbar or 65-66 mm Hg (Lyubarskiy, 1963; Firsov, 1966)).

However, there is twice as much gas above the surface of Mars than there is at an altitude of 17 km above the Earth, since it is necessary to have more gas to obtain the atmospheric pressure which exists on Mars due to its lower mass.

According to other information, the density of the Martian atmosphere is much less: according to the findings of devices that were mounted aboard the "Mariner-4" probe, a pressure was calculated equal to 7-4 mbar (Kliore et al., 1965). No oxygen was found in the atmosphere of Mars, although indirect data indicate its presence. Apparently, the amount of it does not exceed 0.15% of that on Earth. There is a basis for considering that free oxygen existed earlier in the atmosphere of Mars in large amounts but is now combined with the rocks of the planet. /82

In conjunction with the low pressure, the boiling point decreases. At 60 mm Hg, it is 42°, while water will boil at 0° at 4.58 mm Hg. The question arises as to whether protozoa could exist under conditions with such severe temperature variations.

There are many papers devoted to the problem of the sensitivity of protozoa to a reduced content or absence of oxygen in the environment (Kitching, 1939; Brand, 1951). It has been shown that paramecia can live under anaerobic conditions for periods ranging from several hours to several months. However, according to other data, paramecia will die in 10 seconds in gas which is scrubbed of oxygen by means of an alkaline solution of pyrogallol. We observed that the paramecia withstood conditions without oxygen when the oxygen content in the medium was gradually reduced and when the medium was gradually saturated with it.

Reactions to anaerobic conditions depend on temperature, phase of development and the age of the culture of organisms. Obviously, the different results obtained by various authors are explained by these reasons, and also by the fact that when the air is replaced by other gases (hydrogen, nitrogen) the calculated quantity of oxygen may not always be contained in them.

A great deal of information is available in conjunction with infusoria of the species *Colpoda*. Free-swimming (active) forms such as *Colpoda diodenaria* will die at a low partial pressure of oxygen (Taylor, Strickland, 1936); when the partial pressure of oxygen is reduced to 1/10 of its content in air and the air pressure is 15 mm Hg, Colpoda are able to excyst, but they cannot survive when the air is completely replaced by nitrogen for more than 25 hours (Brown, 1939).

Data on simultaneous action of rarefaction of air and low negative temperatures are very rare. Thus, Becquerel (1936) kept the following at the temperature of liquid helium for three months: water plants, rhizopoda, infusoria, rotifera and tardigrada; these objects were dried in vacuum over barium oxide, and the animals survived under these conditions.

Annear (1956) kept the flagellate *Stigomonas oncopelty* dried in a 2% solution of glucose for 12 months in a vacuum at temperatures of 4 and 20°. After this treatment, these flagellates multiplied readily.

According to the data of Taylor and Strickland (1936), dry cysts of /83
Colpoda cucullus will survive in a high vacuum ($1 \cdot 10^{-5}$ atm) when frozen in liquid nitrogen just as well as the controls; 71 and 76% survived, respectively. All of these data applied to dried organisms.

We did not find any information in the literature on the influence of various degrees of rarefaction of the air or other gases on active colpoda; we still do not know how the dormant cysts of protozoa will survive in a moist medium when frozen in a rarefied atmosphere or other gases. According to Siegel et al., (1965) the resistance of plants to low temperature is increased with an insignificant content of oxygen in the atmosphere (2% O_2, 98% Ar).

The purpose of this study was to investigate the influence of rarefaction of an atmosphere of air and nitrogen on the survival and multiplication of *Colpoda maupasi* and the resistance of its cysts to cooling under low atmospheric pressure conditions and hypoxia.

Material and Method

The experiments were performed with infusoria (*Colpoda maupasi*) obtained from lichens collected on the Tseysk Glacier (Northern Caucasus, altitude 2,000 meters above sea level) in 1963. The infusoria were cultured on a mineral medium (Lozina-Lozinskiy, 1948) at a temperature of 22-24°. As the source of nourishment, bacteria of the species *Bacillus subtilis* were supplied.

The experiments were performed with active infusoria (trophonts) and dormant cysts in a Lozina-Lozinskiy medium and cysts that had been dried for 24 hours on filter paper. Each experiment involved the study of from 12 to 50 active infusoria, set out in individual microaquaria each containing 0.2-0.3 ml of medium. The experiments were repeated 3-4 times.

This method made it possible to determine easily the survival and the rate of multiplication. The dormant cysts with liquid medium were tested individually with 8 to 42 repetitions, in groups of 10 to 50 cysts for 5 to 10 repetitions or 100 to 500 cysts for 3 to 4 repetitions in various series of tests. Each series was repeated from 3 to 11 times. In the experiments with dried cysts, from 375 to 2,500 infusoria were used in each test with 3 to 7 repetitions.

Rarefaction of the air with a residual pressure of 10 mm Hg was accomplished in a microanaerostat with a capacity of 2.5 liters in 5 to 10 minutes, or in 50 to 60 minutes. In many of the series of experiments, a rarefaction equal to 5 mm Hg was produced in ampoules with a capacity of 3.0 and 4.0 ml in 1 to 3 minutes, and the ampoules were then sealed, with the degree of rarefaction being recorded at the same time by a mercury manometer. To observe the effect of reduced pressure beneath the microscope, the infusoria were placed in a hanging drop in a Karel vessel or in an ampoule containing /84
0.5 ml of medium.

Another series of tests was performed in a special apparatus called the "photostat". In the latter, it was possible to simulate more natural conditions of the environment. In the vacuum chamber of the "photostat" (with a volume of 18 liters), a given atmospheric pressure equal to 5 mm Hg was

produced with constant exchange of gas. The pressure was recorded automatically and recorded on the strip chart of the device. In the experiments described below, air, technical nitrogen containing up to 1% oxygen and purified nitrogen containing 0.0005% oxygen at rarefactions of 45, 15, 10 and 5 mm Hg were passed through the chamber of the "photostat" in which the infusoria were located at a rate of 240 ml per minute. Variations in pressure reached 2.5 mm Hg.

In order to avoid significant evaporation of the medium, the microaquaria containing the infusoria were placed in Petri dishes or crystallizers on whose bottoms water had been poured; the dishes were covered loosely with covers and the crystallizers were covered with disks made of plastic which did not prevent aeration. The effect of rarefied atmosphere on infusoria was studied at temperatures of 20 ± 2° and at 6-8°.

The experiments with anaerostats were continued depending on the survival at different atmospheric rarefactions, sometimes lasting more than a month at pressures of 15 mm Hg or more. The duration of the experiments in the "photostat" did not exceed two days, since the medium dried out during longer periods.

The cysts were cooled in a low-temperature refrigerator to -30°, with the cysts being placed in watchglasses in the anaerostats in dry ice (-79°), on watchglasses or in ampoules with rarefied atmosphere, in liquid nitrogen (-196°), in ampoules and test tubes. Infusoria were frozen in water at +30°; the atmospheric pressure was established in the anaerostat in the course of several seconds while it required 5 to 7 minutes in the chamber of the "photostat".

The influence of these conditions was evaluated on the basis of the survival of the infusoria, the ability to excyst and the rate of division, i.e., the number of daughter infusoria from one individual with daily transfer, or all of the offspring from one infusorian were counted for each day without transfer. As controls, we used the infusoria which were not subjected to the action of rarefaction and low temperatures or were subjected to the action of only one of these factors.

Effect of Rarefaction of the Air in Hermetically Sealed Vessels

In the anaerostat, the survival of active infusoria changes as a function of the rarefaction of the air above the liquid medium. It is apparent from the data in Table 1 that as the rarefaction in the anaerostat increases, the death of trophonts (in two days) increases (at a temperature of 18 to 20°).

Threshold rarefaction is at approximately 15 mm Hg; at pressures below this level, there is a significant decrease in survival.

TABLE 1. SURVIVAL OF ACTIVE *Colpoda maupasi* AT DIFFERENT AIR PRESSURES FOLLOWING EXPOSURE FOR TWO DAYS (IN % OF CONTROL)

Pressure, mm Hg.	Number of Experiment								
	I	II	III	Avg.	I	II	III	IV	Avg.
	Anaerostat				"Photostat"				
5	0	0	0	0	80.0	92.0	100	84.0	89.0
10	68.0	56.0	13.0	45.7	97.9	100	--	--	98.9
15	100	100	100	100	100	96.0	100	--	98.5
45	100	100	100	100	100	100	100	--	100

At 10 mm Hg, the trophonts survive satisfactorily for several days; after two days, the number of living animals is about 40% on the average, but after three days all of the infusoria are dead. In a number of experiments, rarefaction to 10 mm Hg produced 100% deaths on the first day. Rarefaction of the air to 5 mm Hg killed the active infusoria that were in the hanging drop in all experiments within 50 to 90 minutes, but in 2.5 to 3 hours in an ampoule containing 0.5 ml of liquid medium. At this reduced pressure, the infusoria were initially seen to slow down their movements and then ceased progressive movement, curled up and after turning in one position, broke up. Before this took place, the contractile vacuole increased significantly, taking up almost all of the cell.

Survival of the trophont was possible if rarefaction to 5 mm Hg was performed at a temperature of 6-8°. Under these conditions, the infusoria initially swelled somewhat, and slowed down their movements but did not split.

When the infusoria were restored to original conditions of temperature and pressure, their condition returned to normal. /86

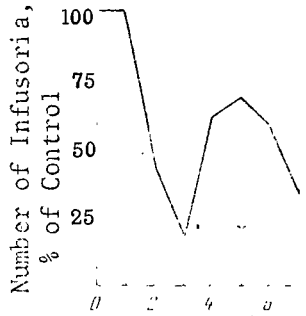

Figure 1. Rate of Division of *Colpoda maupasi* at an Air Pressure of 15 mm Hg.

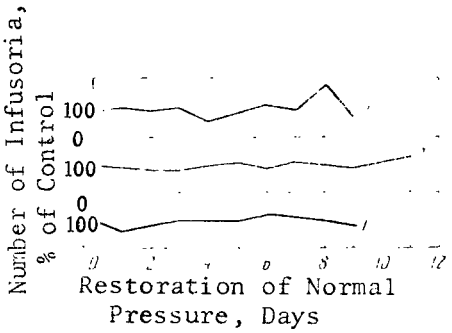

Figure 2. Rate of Division of *Colpoda maupasi* Following Action of Atmospheric Air Rarefied to 15 mm Hg.

1-3 = after 1, 2 and 45 days under rarefaction, respectively.

In a liquid medium with bacteria and in air atmosphere rarefied to 15 mm hg, infusoria can survive for a long time, at least 1-1.5 months. However, the division rate of the infusoria under these conditions is lower as compared to the rate of multiplication of the controls under normal atmospheric air pressure (Figure 1). It is particularly reduced on the second or third day following imposition of the rarefied atmosphere. On the third day, the rate of multiplication of the experimental animals amounted to a total of 18% of the rate of the controls. The reduction of the difference in the rate of division of the experimental and control infusoria on the fifth day of division is explained most likely by the fact that at this time the encystation of the controls was beginning, while the infusoria in the rarefied atmosphere continued to multiply. In addition, the number of active infusoria in the experimental groups decreased in conjunction with encystation. /87

A drop in the rate of division with rarefaction of the air was observed in experiments involving *Colpoda maupasi*, found in soil from a permafrost zone on the slopes of Mount Razvalka (Northern Caucasus, Mineral'nyye Vody Rayon). These infusoria withstood rarefaction of the atmosphere to 15 mm Hg for three days, but their rate of division decreased by a factor of three relative to the controls, and these infusoria subsequently died.

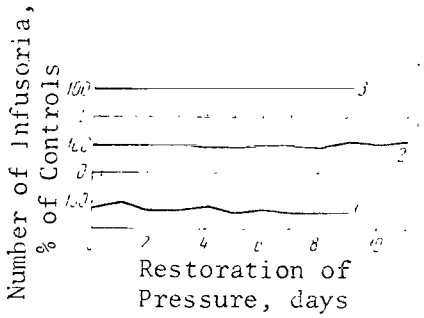

Figure 3. *Colpoda maupasi* Following Restoration of Normal Atmospheric Pressure of the Air. 1-3 equals after 1, 2 and 45 days under rarefaction, respectively.

Following restoration of normal atmospheric pressure of the air, the survival and division rate of the infusoria subjected to the action of rarefaction became the same as for the controls (Figures 2 and 3).

In vessels with less capacity -- ampoules with volumes from 4 to 0.3 ml of medium -- at the same pressure of 15 mm Hg, infusoria survived much less satisfactorily (35%). The rarefaction rate has no effect on the survival of trophonts if, for example, the pressure varies during five minutes or one hour.

The cysts resulting from the multiplication of *Colpoda maupasi* in a rarefied atmosphere up to 15 mm Hg survived and completed their development. At lower pressures they died like the trophonts.

TABLE 2. EXCYSTATION OF DORMANT CYSTS OF *COLPODA MAUPASI* AT VARIOUS AIR PRESSURES IN THE ANAEROSTAT (IN % OF CONTROL VALUE). DURATION OF EXPERIMENT -- 2 DAYS

Number of Experiment	Excystation of Cysts Under Pressure, mm Hg.=		
	45	15	10
1	100	100	7.4
2	100	36.4	8.0
3	100	48.1	3.3
4	--	100	--
5	--	72.0	--
Average	100	80.9 ± 17.1	6.2 ± 1.9

The dormant cysts withstand an atmospheric pressure of 5 mm Hg and probably lower temperatures as well both in a liquid medium and dried on filter paper. However, a rarefied atmosphere of air above a liquid medium containing dormant cysts affects their excystation (Table 2). At rarefactions below 5 mm Hg, there is no excystation of *Colpoda maupasi*.

Action of Rarefied Atmospheric Air Flow in the "Photostat" /88

Completely different results were obtained in the "photostat" chamber with constant throughput of air. The data shown in Table 1 indicate that active infusoria survived well both with rarefaction of the air to 15 and 10 mm Hg and with rarefaction to 5 mm Hg (regardless of whether the temperature was 8 or 20°). It was found that 79.2% of the dormant cysts of these infusoria excysted even with rarefaction to 5 mm Hg of air. Under these conditions of rarefaction and at a temperature of 20°, infusoria multiplied with the same speed as in the control group.

Effect of Rarefied Nitrogen in the Anaerostat

To answer the question as to whether it is only a shortage of oxygen that affects the infusoria when the air is rarefied or whether their decrease in pressure is of some significance, experiments were organized in which the air was replaced by nitrogen.

For these experiments, we used nitrogen which contained 1 and 0.0005% oxygen. Initially, the air was pumped out of the anaerostat to provide the minimum possible amount which according to the reading of the mercury manometer was about 3-5 mm Hg; the chamber was then filled with nitrogen to normal atmospheric pressure, and then rarefield to 45, 15, 10 and 5 mm Hg. The experiments showed that at a pressure of 15 mm Hg or more in an atmosphere of nitrogen containing 1 and 0.0005% oxygen, infusoria will survive and multiply for two days in practically the same fashion as in an atmosphere of air at the same pressure. At high levels of rarefaction -- up to 10 and 5 mm Hg -- the infusoria begin to die on the first day (Table 3). They cannot excyst under these conditions.

In ampoules with a volume of 15 ml at a rarefaction of 15 mm Hg nitrogen with 1% oxygen does not kill infusoria in two days in 9 samples out of 10, at 10 mm Hg in 2 samples out of 10 and at 5 mm Hg all of the infusoria die.

Effect of Rarefied Circulating Nitrogen Atmosphere in the "Photostat"

In contrast to the experiments in the anaerostat, infusoria survive well and multiply in an atmosphere of technical nitrogen rarefield to 10 and 5 mm Hg

in the vacuum chamber of the "photostat". Approximately 67% of the infusoria will excyst at a pressure of 5 mm Hg.

With a constant interchange of purified nitrogen for two days, the same high percentage of insuforia (92%) will survive as in an atmosphere of technical nitrogen at pressures of 10 mm or more. However, in contrast to the insuforia that were in nitrogen containing 1% oxygen, the rate of division is much lower (Table 4).

The insuforia themselves were characterized by slightly smaller size and reduced movement. Aproximately 80% of the infusoria excysted. Consequently, with a content of 0.0005% oxygen in the atmosphere and with reduced pressure amounting to 1/150 of normal atmospheric pressure, aerobic insuforia (*Colpoda maupasi*) can exist, although the conditions are classified as borderline. The existence of unicellular organisms at such negligible oxygen levels as in /91
purified nitrogen and low pressure is possible thanks to the continuous replacement of the gas in the vacuum chamber of the "photostat".

Effect of Severe Cooling on the Dormant Cysts of *Colpoda maupasi* in the Normal and Rarefied Atmosphere

According to the data in the literature, infusoria of the species Colpoda *(Colpoda cucullus)*, while in the dormant cyst stage, can withstand the temperature of liquid nitrogen both in the dried state and in a fluid medium (Taylor, Strickland, 1936). In a liquid medium, according to the statements of these authors, insuforia will survive only with rapid cooling (with the temperature dropping at the rate of -200° in several seconds). The experiments of Pigon and Edstrom (1961) showed that dormant cysts of *Colpoda cucullus* are sensitive to temperatures as high as 5°. Death of the organisms at this temperature varies from 10 to 90%.

Cooling of Dormant Cysts at Normal Pressure

It is apparent from the data presented below that the cooling of dormant cysts of *Colpoda maupasi* in a liquid Lozina-Lozinskiy medium to -30° did not cause their death. After one or two days at a temperature of -30° 100% of the infusoria excysted.

At a cooling Temperature, °C	Survival of cysts (in % of controls) at atmospheric pressure equal to	
	760 mm Hg	5 mm Hg
-30	100	97.2 ± 3.6
-79	67.7 ± 27.6	87.5 ± 12.5
-79, -196	66 ± 5.8	82.3 ± 16.8
-196	0	0

Under these conditions, cooling was slow and crystallization of the water inside the cells did not take place. The high survival rate was also observed during cooling in dry ice: it varied between 100 and 30%. Since the cysts were in ampoules containing a liquid medium, the cooling rate down to -79° was also low -- about 3° per minute. At this rate, the cysts could dehydrate and intracellular freezing did not take place. Following rapid cooling of the ampoules containing the dormant cysts in liquid nitrogen (-196%), no encystation was observed, at least for two days.

If the cysts were initially cooled to -79°, (for periods of 30 and 60 minutes), and then placed in a chamber with a temperature of -196°, the percentage of excysted infusoria was the same as following a single cooling to -79°. /92

Direct observation and photography of the cooling process of the cysts in the field of a luminescence microscope (using incident light) supported the suggestions that were made to explain the data on the effect of the cooling rate. During slow cooling of the preparation containing the cysts to the temperature of dry ice, there was a gradual shriveling of the cysts: none of the dark cells which are formed during intracellular crystallization of water (Lozina-Lorinskiy, 1963, 1966) were observed. There was no rupture of the cell envelope. After the cell thawed, it again assumed a round shape. With rapid freezing in liquid nitrogen, it is possible to detect the formation of crystals in the cell displacing the cytoplasm and the chromatinic substance, visible thanks to the illumination at the periphery of the cell. Rupture of the envelope was seen to occur in many cells at the time of thawing.

TABLE 3. SURVIVAL OF ACTIVE *COLPODA MAUPASI* AT VARIOUS NITROGEN PRESSURES FOLLOWING TWO DAYS OF EXPOSURE (IN % OF CONTROL)

Pressure, mm Hg.	Anaerostat, Number of experiment													
	I	II	III	IV	V	VI	VII	Average	I	II	III	IV	V	Average
	Nitrogen, 99.0%								Nitrogen, 99.9995%					
5	0	0	0	0	—	—	—	0	0	0	0	—	—	0
10	76,7	30,0	0	36,7	63,3	70,0	0	39,5	76,7*	51,1	0	0	0	25,5
15	80,0	90,0	100	—	—	—	—	90,0	96,7	89,6	100	93,3	—	94,4
45	95,9	—	—	—	—	—	—	95,9	95,3	63,3	96,7	—	—	84,4

Pressure, mm Hg.	"Photostat", Number of experiment										
	I	II	III	IV	Average	I	II	III	IV	V.	VI
	Nitrogen, 99.0%					Nitrogen, 99.9995%					
5	100	91,3	80,0	92,0	90,8	100	100	26,5**	20,0***	52,0***	59,7
10	97,9	100	—	—	98,9	100	100	100	—	—	100
15	100	88,0	88,6	—	92,2	—	—	—	—	—	—
45	100	100	100	—	100	—	—	—	—	—	—

* On the first day

** In ampoules

*** 0 equals negative results, program interrupted, no experiment.

Commas indicate decimal points.

Table 4. RATE OF DIVISION OF *Colpoda maupasi* AT DIFFERENT AIR AND NITROGEN PRESSURES AT A TEMPERATURE OF 18-20° (RELATIVE TO CONTROLS)

Atmosphere	No. of exper.	Classification by Pressure, mm Hg.		
		15	10	5
		Anaerostat		
Air	1	0,7±0,3	0,02±0,008	—
	2	1,4±0,5	0,30±0,10	—
	3	0,5±0,16	0,06±0,03	—
	4	0,2±0,1	0,11±0,07	—
	5	0,08±0,005*	0,21±0,06	—
Nitrogen, 99.0%	1	0,11±0,04	0,20±0,08	—
	2	0,57±0,22	0,10±0,03	—
Nitrogen, 99.9995%	1	1,30±0,83	0,45±0,15	—
	2	1,50±0,69	0,44±0,23	—
	3	0,56±0,05	—	—
		"Photostat"		
Air	1	0,30±0,03	0,50±0,26	1,9±0,8
	2	1,70±0,5	1,30±0,70	1,3±0,3
	3	1,30±0,7	0,5±0,3	—
	4	—	1,0±0,4	—
Nitrogen, 99.0%	1	0,4±0,25	1,30±0,09	1,7±0,4
	2	0,5±0,00	0,30±0,09	1,9±0,6
	3	0,5±0,18	—	—
	4	0,5±0,16	—	—
	5	0,5±0,14	—	—
Nitrogen, 99.9995%	1	—	—	0,3±0,2

*In ampoules, 4.0 cc.

Commas indicate decimal points.

Freezing Dormant Cysts in a Rarefied Atmosphere

Dormant cysts were subjected to cooling in a liquid medium (Lozina-Lozinskiy) and dried on filter paper for a day at a temperature of 23° in air rarefied to 5 mm Hg.

After the cysts were cooled at low pressure to -30°, the infusoria in the liquid medium excysted (97.2% in comparison to the controls). The percentage of infusoria that excysted from cysts cooled to -79° was somewhat less, as was

the case with stepwise cooling to -196°. The survival rate with severe cooling under conditions of rarefied atmosphere was 20% higher on the average than at normal pressure (see above). However, this difference was not statistically reliable.

The dried cysts, as we would expect, have about the same resistance to cooling under conditions of low pressure as cysts from a liquid medium: following cooling to -30°, 85.7 ± 7.9% excysted, to -79°, 8.6 ± 11.1% and to -196°, 90%, respectively.

Evaluation of the Results Obtained

The question of the composition and density of the gaseous medium in which unicellular organisms can exist is of gneral biological interest and significant for several problems of exobiology. In particular, it is still not clear what the minimum quantities of oxygen in the atmosphere and how great the rarefactions are which will allow the existence and multiplication of protozoa that belong to /93 the aerobic and freely mobile forms.

The data contained in the literature on the respiration of protozoa and their oxygen requirements indicate the existence of anaerobic forms in nature, as well as forms which change over completely under certain conditions to the glycolytic form of metabolism and become anoxiobionts temporarily. The study of the reactions of protozoa to a reduced content or absence of oxygen in the medium and their ability to withstand anoxia or hypoxia have been studied by various methods. On the basis of the use of inhibitors which suppress the various branches of energetic metabolism, it was found that there was a need for certain respiratory enzymes and activity of enzymes depending on the gas composition of the medium.

It is difficult to evaluate the possibility of anaerobiosis of animals from a population of biotopes in which there is very little oxygen or only traces of it, since we do not know whether or not these organisms exist only due to anaerobic metabolism or whether they use traces of oxygen. A direct method of determining the viability of organisms in a medium without oxygen or with reduced content of the latter is to place them in hermetic vessels from which the gas is removed,

replacing it by some inert gas or by absorbing the oxygen by means of chemical absorbers.

This method, however, has several difficulties and shortcomings. It does not take into account the change in the composition of the atmosphere in the closed vessel with organisms in which remnants of oxygen may be abosrbed completely by cells and where gases may develop as the result of metabolism. In gases which have been chemically scrubbed of oxygen, for example with the aid of an alkaline solution of pyrogallol, there are traces of oxygen which can maintain the vital activity of aerobes for a short time. Therefore, a short stay (several hours) of organisms and cells under such conditions will have no effect on their capacity for anaerobiosis. We can assume that following absorption of the traces of oxygen the cells will die as a result of the anaerobic conditions which develop. Consequently, the direct method of ordinary study of sensitivity of organisms to a shortage of oxygen in a closed environment, for example in anaerostats and vessels of other types, cannot provide an exact explanation of the need of cells for oxygen and will not demonstrate their capacity for anaerobiosis in short experiments. Moreover, experiments with cultures of organisms in hermetically sealed chambers are difficult to compare with one another, since the amount of residual oxygen that remains after their removal depends on the volume of the vessel and the amount of oxygen required depends on the number of cells and their physiological condition, which changes during incubation. Hence, to solve the problems with which we are faced, these problems cannot be considered satisfactorily.

The presence of oxygen on Mars has not been proved, but /94
it is assumed that it is found in amounts which are approximately 1,000 times less than in the atmosphere of the Earth. The question of whether or not aerobic microorganisms and lower life forms could exist under these conditions cannot be answered by means of studying their resistance to a shortage of oxygen in closed chambers like anaerostats. In this connection, a continuously renewed atmosphere was created in a specially built chamber called the "photostat", with a set pressure imitating an open system like the atmosphere on some planet.

This work demonstrated the ability of free-living aerobic infusoria (*Colpoda maupasi*) not only to survive but to multiply under conditions in which

the air was rarefied to 15 mm Hg in a hermetically sealed chamber (micro-anaerostat) and to 5 mm Hg in the "photostat". The oxygen content in these chambers reached 0.5 and 0.16%, respectively, of the amount contained in normal atmospheric air. With rarefaction of the air to 15 mm Hg in a sample containing 0.3 ml of liquid medium, we found 0.2 microliters of oxygen. In the opinion of Adolph (1929) and Amberson (1928), the ability of small infusoria to survive with a low content of oxygen in the medium is due to the low level of consumption of the latter. However, a reduction of the rate of division with a rarefaction of the air to 15 mm Hg in a hermetically sealed chamber (anaerostat) or in a sealed ampoule indicates a decrease in the intensity of the cellular metabolism.

According to the data presented by Pigon (1959), one type of infusoria (*Colpoda cucullus*) uses $10\text{-}20 \cdot 10^{-5}$ microliter of O_2 per hour. If these data are applied to *Colpoda maupasi*, then in a sample of 0.3 ml one infusorian can live and multiply for three to five days with a constant maximum utilization of $20 \cdot 10^{-5}$ microliter of O_2. In the encysted state and at low temperatures, the utilization of oxygen decreases by a factor of 10 to 20 or more. In this connection, the possible lifetimes of infusoria in rarefied air are increased when the latter is constantly changed.

Hence, the survival and multiplication of *Colpoda maupasi* in an atmosphere of air rarefied to 5 mm Hg can be given a theoretical basis.

In the literature, the viability of animals at various levels of rarefaction is evaluated from the standpoint of oxygen insufficiency. Our experiments in the anaerostat with replacement of air by nitrogen (containing a trace of oxygen -- 0.0005%) appeared to indicate a certain role being played by reduced pressure. Under conditions of rarefaction of the air below 5 mm Hg, infusoria (*Colpoda maupasi*) will not survive at 20°, but if the pressure is increased to 15-10 mm Hg of chemically pure nitrogen, then about 100% of the infusoria will survive and multiply.

However, in a chamber where the air or the nitrogen is changed during the experiment, the infusoria will survive and will excyst from their dormant cysts even at rarefactions of 5 mm Hg and a negligible oxygen content in the nitrogen. /95

The survival and multiplication of infusoria in an atmosphere of "pure" nitrogen, containing a maximum of 0.0005% O_2 at a pressure of 5 mm Hg, indicate not only the low consumption of oxygen but also the ability of the cells to extract the traces of this gas. Consequently, we cannot always speak of the anaerobic nature of unicellular organisms if they exist in "pure" nitrogen or in an atmosphere of some other gas which has not been completely scrubbed of oxygen.

The conditions of a fixed atmosphere in the "photostat", forming an open system, imitate the possible exchange of gases from the surface of the planet Mars to a greater degree than the experiments which are performed with organisms in hermetically sealed systems.

On the basis of the data obtained, we can assume that low pressure and traces of oxygen in the atmosphere of Mars cannot serve as a hinderance to the existence of certain terrestrial animals resembling the soil infusoria *Colpoda maupasi*.

According to the information from a number of authors, the swelling of the cells of invertebrate animals under anaerobic conditions is caused by a disruption of osmoregulation (Vinberg, 1948). In the case of trophonts of *Colpoda maupasi* under conditions of atmospheric rarefaction to 5 mm Hg, the rounding and swelling can also be explained by an increase in the permeability of the organism to water and disruption of osmoregulation. This is indicated by the sharp increase in the size of the contractile vacuole.

Data in the literature indicate that aerated cells of facultative anerobes (yeast organisms, the bacterium *Escherichia coli*, etc.) are more resistant to severe cooling than the same cells in a medium without oxygen in it (Rumyantseva, Tribis, 1965; Nei et al., 1967). Therefore, it was interesting to study the resistance of Protozoa to the process of cooling with a reduced content of oxygen in the air and low pressure and with traces of oxygen in the atmosphere of nitrogen, especially since a combination of low temperatures and a neglibile content of oxygen in the atmosphere is characteristic of Martian conditions.

The experiments showed that an insignificant oxygen content and low pressure have almost no effect on the resistance of the infusoria *Colpoda maupasi* to severe cooling.

The ability of aerobic infusoria to exist and multiply at a very low atmospheric pressure, with the atmosphere containing a negligible amount of oxygen, under conditions of sharp diurnal variations and temperature, the ability to rapidly change from the active state to a dormant state and vice versa, the resistance of the dormant cysts to severe cooling and freezing, as well as drying, the high resistance to ultraviolet rays all indicate that the physical conditions on the planet Mars do not exclude the possibility of unicellular animals existing there.

In conjunction with the small size of certain unicellular and multicellular animals, it is not impossible that they could be transferred (like microorganisms) to other planets in the event of insufficient attention being given to measures for sterilization of spacecraft. /96

Conclusions

1. Aerobic infusoria (*Colpoda maupasi*) may live and multiply at atmospheric pressure from 45 to 15 mm Hg in an atmosphere of air or nitrogen, containing 1 or 0.0005% oxygen. The rate of division at a pressure of 15 mm Hg is reduced. When the air or nitrogen is rarefied to 10 mm Hg, there is a considerable decrease in the survival rate of the trophonts and an absence of multiplication; at 5 mm Hg, all of the animals will die. The dormant cysts will survive at these pressures, but the animals can excyst only at atmospheric rarefactions above 15 mm Hg.

2. In a special vacuum chamber, the "photostat", in which a closed atmosphere was created during an experiment lasting several days and in which low pressure was maintained automatically, infusoria multiplied and excysted not only at pressures of 10 to 15 mm Hg, but even at 5 mm Hg in air or nitrogen with addition of 1 and 0.0005% oxygen.

3. Dormant cysts of *Colpoda maupasi* in a liquid medium can withstand severe cooling to - 79 and - 196° under conditions of slow cooling.

4. Resistance to severe cooling of cysts under conditions of rarefaction of the air to 5 mm Hg is no less than at normal atmospheric pressure.

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INFLUENCE OF A SET OF EXTREMAL FACTORS ON BIOLOGICALLY ACTIVE SUBSTANCES

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ABSTRACT. Principal differences between physical conditions existing on Mars and on Earth are reviewed. Martian conditions are simulated and their influence on proteins is examined using RNA-ase as an example. Particular attention is given to the effect of low temperatures and strong ultraviolet radiation.

The conditions of a medium which go beyond the limits that are normal for the organism are called extremal (Lozina-Lozinskiy, 1966). They include low and ultralow temperatures, high and low pressures, doses of ionizing and ultraviolet radiation that exceed the natural background. A study of the influence of these factors on the organism, the cell, its components and biologically important substances is of particular interest for recognizing the boundaries of life and solving the question of its propagation beyond the limits of the Earth.

Of all the planets in the Solar System, Mars is most similar to Earth and is therefore most suitable for development of life.

The principal differences in the physical conditions on Mars from those on Earth are the following:

1) much higher ultraviolet radiation with wave lengths of 200-300 nm; 2) lower temperature and sharp changes in the course of a day; 3) lack of oxygen in the atmosphere; 4) an atmospheric pressure of 0.01 atm, according to data obtained by the "Mariner"; 5) insignificant content of free water.

In this paper, we have dealt with the effect of these factors on a protein with a well-known structure -- the enzyme RNA-ase. We studied the manner in which it is affected by low temperatures as well as ultraviolet radiation in conjunction with other factors (temperature, pressure, gas composition, solvent).

The data we obtained on the biological effectiveness of the action of ultraviolet light is a function of the attendant factors was then tested under simulated Martian conditions.

Method /98

In the experiments, we used a crystalline preparation of RNA-ase from the firm of Reanal (Hungary). The activity of the enzyme was evaluated on the basis of its biological activity determined spectrophotometrically according to Anfinsen et al., (1954).

To clarify the nature of the changes in the molecule of the enzyme under the influence of low temperatures, solutions of RNA-ase were studied on an oscillographic automatically recording polarograph (P-04).

Experimental solutions were prepared in doubly distilled water or mixtures of water with glycerine, with the content of the latter varying from 60 to 99% (in volume). The concentration of the enzyme in the investigated solutions amounted to 2 mg %.

The solutions were frozen in volumes of 1 ml at temperatures of -30, -70, -189 and -269°. The first two temperatures were attained in the ultrathermostat (U-6), which is capable of ensuring attainment of low temperatures. To freeze the solutions at ultralow temperatures of -196 and -269°, the samples were placed in liquid nitrogen or helium, respectively. Samples were thawed in air at a temperature of 20° in 30 minutes (slow thawing).

The solutions were irradiated with integral sources of ultraviolet radiation -- the PRK-2 and SVD-120A lamps. The intensity of the ultraviolet radiation (λ = 240-300 nm), as determined by the UFI-65 dosimeter, amounted to $1 \cdot 10^3$ ergs/cm^2/sec and $13 \cdot 10^3$ ergs/cm^2/sec for the PRK-2 and SVD-120A lamps, respectively, at the points where the samples were exposed.

In experiments involving the study of the influence of the temperature of enzyme solutions on their resistance to ultraviolet radiation, exposure was performed in a temperature range from +10 to -180°.

At ultralow temperatures, the solutions were irradiated in a cryostat (built in the GDR) with the aid of a special device which we built.

The complex of Martian conditions -- characteristic variations of temperature, insolation, pressure and specific gas composition were produced in a chamber referred to as the "artificial Mars", which was built in our laboratory. The change of temperature in the chamber during the course of a day was performed according to values that are given below on the basis of data regarding the temperature on Mars (Vokuler, 1956; Kliore et al., 1965). This daily temperature program provides a good reflection of the basic characteristics of the Martian temperature -- its low values (as low as -64°) and considerable daily variations (92°). The source of ultraviolet light in the chamber was a PRK-2 lamp.

The diurnal cycle of illumination was provided by an automatic device (KEP-12U). According to the data obtained with the "Mariner", the intensity of the ultraviolet light at some distance from Mars in the wave length region /99 from 240 to 280 nm was given a value of $2 \cdot 10^3$ ergs/cm^2/sec (Horowitz, 1966).

In our experiments, the intensity of the UV in the same region of the spectrum at the points where the samples were exposed in the "artificial Mars" chamber was equal to $1 \cdot 10^3$ ergs/cm^2/sec. The source of UV-rays was switched on when it was 0600 hours on the "artificial planet" and switched off 12 hours later.

An atmosphere of different composition could be created in the chamber (pressure up to 0.01 atm). The gas mixture was prepared before hand in a mixing tank and fed into the chamber from the latter after the air had been removed. The regulation of the gas pressure in the chamber was performed by an automatic system of manometers and mercury relays.

In this study, the gas pressure in the chamber was 0.1 atm. Gaseous nitrogen was carefully scrubbed of oxygen by passing it through a system of absorbers.

The chamber was sealed after the vessels were placed in it and the air was pumped out. As soon as the pressure in the chamber reached 0.1 atm, nitrogen was admitted to it until the final pressure equal to 1 atm was reached. Then the gas was removed and the chamber was refilled with a new batch of nitrogen. This procedure, "washing out of the chamber," was performed four times.

As soon as the final gas pressure of 1 atm was established in the chamber, the temperature regulating system was switched on. The UV source was switched on as soon as the desired temperature was reached in the chamber.

The samples that were subjected to the influence of Martian conditions without irradiation were placed in the chamber together with those that were irradiated but in glass test tubes and in containers made of aluminum foil.

Results and Their Evaluation

Low temperatures do not have as much of an effect on enzymes as do high ones. Usually the changes that are caused by low temperatures in a molecule of enzyme are reversible and in the case of a single freezing the isolated enzymes can be stored for a long period of time without inactivation at ultra-low temperatures. We know, however, that with repeated freezing and thawing of enzyme solutions, many of them are inactivated (Kiermier, 1958; Shikama, Yamasaki, 1961). We did not find any data on the influence of low temperatures upon RNA-ase.

Experiments dealing with the effect of various temperatures on solutions of RNA-ase were performed with single or repeated freezing, keeping the solutions in this state for various periods of time and then thawing them slowly.

The study of the enzyme solutions that were frozen at temperatures of /100
-30, -70, -196 and -269° showed that their being kept at these temperatures for long periods of time (from 1 hour to 7 days) had no effect on the activity of the enzyme following thawing.

Other results were obtained with multiple freezing of the investigated solutions. Below we have listed data on the activity of the enzyme (in %, as an average of four parallel experiments) with fivefold freezing and thawing of solutions of RNA-ase in water and in glycerine.

Temperature, °C	In Water	In Glycerine (60%,V)
- 30	82	100
- 76	100	100
- 196	73	100
- 269	71	100

Note. Enzyme activity of solutions of RNA-ase prior to exposure was taken as 100%.

From these results it follows that freezing of solutions at ultralow temperatures (-196 and -269) leads to inactivation of 30% of the enzymes. This effect also takes place at -30°, but is absent at -76°. The dependence of the effect on temperature obviously has something to do with the different structure of ice, the nature of the formation of which is determined by the rate and temperature of freezing (Blackman, Lisgarten, 1957).

It follows from the average data from five parallel experiments listed below that inactivation of the enzyme increases with increasing number of freezings of the solution at -196° with subsequent slow thawing.

No. of Exposures	Inactivation, %
1	0
3	21
5	29
10	41
15	75

To determine the nature of the denaturation of RNA-ase with repeated freezing and thawing, we performed polarographic investigation of a solution of enzymes following tenfold exposure. In Braichka reagent, with a final protein concentration equal to 0.25 mg/ml, we obtained typical double "protein waves" for RNA-ase, whose principal characteristics --the height of the phases (h_1 and h_2) and the potentials of the half-waves (E_1 and E_2) --are listed below. /101

Parameters	Prior to Exposure	Tenfold freezing to -196° and Thawing at +20°
E_1, V	1.43	1.43
h_1, mm	11.0	5.0
h_1, %	100	45
E_2, V	1.64	1.64
h_2, mm	13.3	5.5
h_2, %	100	41

From the results that have been presented on the influence of multiple freezing and thawing of a solution of RNA-ase, it is clear that as a result of freezing and thawing the height of the first and second phases of the polarographic wave are considerably decreased, but the potentials of the half-waves remain unchanged.

The decrease in the polarographic wave as a whole without changing the reduction potentials may have something to do with the loss of solubility and the precipitation of a portion of the denatured protein without a change in the polarographic activity of the protein in the dissolved fraction (Ivanov, 1961).

The effect of inactivation through multiple freezing may be removed if substances that prevent crystallization of water are added to the enzyme solution. Such substances may be, for example, polyatomic alcohols. We know that with a 60% (V) content of glycerine in solution, crystallization of water can be prevented.

As follows from the results, listed on page 121, multiple freezing of glycerinized solutions of RNA-ase at the temperatures in question did not lead to a loss of enzymatic activity.

It is no accident that protection of certain terrestrial organisms against the harmful effects of low temperatures is accomplished with the aid of glycerine found in the tissues (Takehara, Asahina, 1960). The possibility cannot be excluded that a similar mechanism of protection against cold can be found in Martian organisms, especially since in the absence of water polyatomic alcohols could partly or completely fulfill the function of a biological solvent.

We also studied the effect of temperature conditions on Mars on the retention of activity of enzymes in water and glycerine solutions. The results of these studies (averages from four parallel experiments) are presented in Table 1.

Below we have listed analogous data on the activity (in %) of solutions of RNA-ase following storage of samples at a temperature of 4°. A comparison of these data (page 121) will show that the insignificant decrease in the activity /102
of the enzyme which is observed under conditions which simulate the diurnal variations of temperature on Mars was slightly less than in the solutions that were kept for the same period of time at a constant temperature of 4°. In glycerine solutions, the enzyme activity did not change in either of the cases in question.

TABLE 1. ENZYME ACTIVITY OF SOLUTIONS OF RNA-ase FOLLOWING THEIR STORAGE UNDER CONDITIONS SIMULATING DIURNAL TEMPERATURE VARIATIONS ON MARS

Martian time, Hours	Temperature, °C	Activity, %	
		Of Water	Of Glycerine (60,V)
1200	+26	100	100
1300	+28	100	100
1400	+14	99.4	100
1800	-15	99.2	100
0000	-35	98.6	100
0600	-60	98.4	100
1200	+26	98.0	100

Exposure, Hours	In Water	In glycerine (60%, V)	Exposure, Hours	In Water	In glycerine (60%, V)
0	100	100	12	96.1	100
1	100	100	18	95.0	100
3	99.6	100	24	92.9	100
6	98.3	100			

These data indicate that sharp diurnal variations of temperature on Mars and the low temperature (down to -60°) do not have an inactivating effect on the enzyme.

The ultraviolet radiation at λ = 300 nm denatures protein. The effect of ultraviolet on RNA-ase is well known (McLaren, 1949; Mueller, 1966a, b). This protein, which contains cystine, is most sensitive to ultraviolet light at λ = 250 nm (maximum absorption of cystine). Under the influence of ultraviolet light, there is an irreversible denaturing of RNA-ase, accompanied by a loss of biological activity and a change in the physical-chemical and optical properties of the protein molecule.

Under the influence of ultraviolet light, we studied the influence of the following factors on the inactivation of RNA-ase: temperature, pressure, absence of oxygen, presence of glycerine in solution.

The use of glycerine as a solvent made it possible to irradiate the solutions of RNA-ase at low temperatures in the form of "transparent gumdrops" and to avoid considerable evaporation of liquid with decreasing pressure. /103

The data (averaged from three experiments) on the inactivation of RNA-ase (in %) during irradiation of the latter in glycerine solutions (99% glycerine + 1% water) with ultraviolet light (from a SVD-120A lamp) at various temperatures are listed below.

Temperature, °C	Residual Activity (in %) with a UV Dose (in erg/cm^2)		Temperature, °C	Residual Activity (in %) with a UV Dose (in erg/cm^2)	
+10	47	0	-80	66	8
0	50	0	-100	88	22
-10	53	0	-130	100	37
-30	49	0	-180	100	44
-50	51	0			

These results demonstrate the interesting fact that the effect of radiation depends on temperature, beginning approximately at -80°. Below this value, the initial photochemical effect of irradiation of the enzyme weakens with decreasing temperature of the irradiated solution. On the other hand, an increase in the temperature from -80 to +10° did not cause a change in the resistance of RNA-ase to UV-rays. The fact that temperatures above zero had no effect on the inactivation of the enzyme by irradiation is explained by the fact that RNA-ase is highly temperature stable.

As far as the increase in the resistance of the enzyme to ultraviolet with decreasing temperature is concerned, this may have something to do with an increase in the number of molecules that are in a relatively stable state. The energy which they absorb is not consumed in association, but is lost through phosphorescence (Setlow, Pollard, 1964).

We have listed below data which represent the average values from four parallel experiments on the inactivation of RNA-ase (in a 99% solution of glycerine) under the influence of ultraviolet light in an atmosphere of air and nitrogen at pressures of 0.1 and 1 atm (temperature 0°).

Pressure, atm.	Residual Activity (in %) with a dose of UV in (erg/cm^2)	
Air	$14 \cdot 10^6$	$22 \cdot 10^6$
1	61.5	39.8
0.1	59.8	40.1
Nitrogen		
1	62.2	39.0
0.1	61.6	37.9

It follows from these results that the effect of inactivation of the /104
enzyme had nothing to do with whether irradiation took place in the absence of oxygen (nitrogen atmosphere) or in the presence of oxygen (atmosphere of air). The effect of ultraviolet on the solutions of enzymes also had nothing to do with the gas pressure above the solution (within limits from 0.1 to 1 atm).

In the following series of experiments, solutions of RNA-ase were tested under simulated Martian conditions. Table 2 lists the data from an analysis of aqueous and glycerine solutions of RNA-ase, which were exposed for various periods of time in the "artificial Mars" chamber, where the total complex of Martian conditions was maintained as much as possible: the intensity of the ultraviolet in the range from λ = 240 to λ = 300 nm amounted to $1 \cdot 10^5$ $ergs/cm^2/sec$, the gas was nitrogen, and the temperature varied during the investigated time intervals from + 26 to - 60°.

TABLE 2. CHANGE IN RNA-ASE ACTIVITY ON EXPOSING SOLUTIONS OF IT IN THE "ARTIFICIAL MARS" CHAMBER.

Exposure, hrs	Temperature C°	UV dose -10^5 $ergs/cm^2$	Activity (in%)	
			Water	Water and glycerine (60%, V)
0.4	-60	9	69.8	100
1	-60	36	50.6	100
2	-20	72	15.3	82.6
3	-14	108	0	74.1
6	+26	216	0	40.8
12	-15	432	0	4.9
18	-35	---	0	0

From the results that were obtained, it follows that after only one hour of exposure of aqueous solutions of RNA-ase in the "artificial Mars" chamber, 50% of the enzyme was inactivated.

The addition of glycerine to the solution (up to 60% by volume) considerably protected the enzyme against the inactivating effect of ultraviolet radiation. With a one hour exposure, the enzyme activity of the tested solution did not change, but after three hours, when all of the enzyme in the aqueous solution was inactivated, the residual activity in the glycerine solution was more than 70%. Consequently, if Martian organisms are able to form polyatomic alcohols of the glycerine type in the course of their metabolism, they not only can protect the cell against the harmful effects of freezing and thawing but in some cases can effect a protective function against brief ultraviolet radiation. Since the minimum temperature in the "artificial Mars" /105
chamber was - 60°, according to the data presented above on the influence of temperature on the resistance of RNA-ase to UV rays, its inactivation in the simulated Martian conditions did not necessarily depend on the diurnal temperature variations. As a matter of fact, as we can see from the results that are listed in Table 2, the nature of the process of inactivation of the enzyme exposed in the chamber does not show any significant dependence on temperature with time.

Consequently, of all the features making up the complex of Martian conditions, only ultraviolet radiation has an inactivating effect on the enzyme studied. The absence in the atmosphere of oxygen has no effect on its action, nor does the pressure, lower than that on Earth, nor do the temperature conditions on Mars have any effect.

In conclusion, the authors would like to take this occasion to express their gratitude to their scientific coworkers, M. I. Yakusheva, for the help she gave in performing the polarographic studies of RNA-ase.

Conclusions

1. A single freezing of solutions of RNA-ase at low and ultralow temperatures has no effect on the activity of the enzyme following thawing.

2. Repeated freezing of aqueous solutions of RNA-ase causes inactivation of the enzyme. The effect depends on temperature. Addition of glycerine to enzyme solutions protects the enzyme against inactivation.

3. Maintenance of aqueous and glycerine solutions of RNA-ase under conditions of simulated diurnal variation of temperature on Mars has no effect as far as inactivation of the enzyme is concerned.

4. Ultraviolet radiation causes inactivation of RNA-ase. The effect has nothing to do with the pressure of the gas above the enzyme solution (from 0.1-1 atm) and the content of oxygen in it. The inactivating effect of ultraviolet light on the enzyme depends on the irradiation temperature. The effect is attenuated when the temperature drops below -80°.

5. Under simulated Martian conditions, the inactivating effect on RNA-ase is produced only by ultraviolet light. The differences that exist between the temperature conditions, pressure and oxygen content in the atmospheres of the Earth and Mars have no effect on the inactivation of the enzyme under the influence of ultraviolet light. Glycerine protects the enzyme satisfactorily against ultraviolet radiation.

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EFFECT OF A HIGH VACUUM ON MICROORGANISMS

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ABSTRACT: Experiments designed to determine the high-vacuum resistance of 15 bacteria strains, 7 fungus species, 5 yeast strains and 5 seaweed species. The survival rates after 72-hour exposures were 100% for *Bacillus mesentericus, Bac. mycoides, Bac. megaterium, B. simplex, Aspergillus oryzae, Asp. terreus* and *Actinomyces globisporus*, 52 to 76% for *Bacillus lini, Bac. subtilis, Bac. mesentericus niger, Micrococcus luteus, Micrococcus aurantiacus* and *Sarcina flava*, 22 to 34% for *Saccharomyces vini* and *Candida tropicalis*, and lower percent for *Pseudomonas fluorescence, Serratia marcescence, Vibrio metchnikovii, Zygosaccharomyces vini, Torulopis aeria, Rhodotorula rubra, Trichothecium roseum* and *E. coli*. It is concluded that a high vacuum of about 10 nanomillimeters Hg would not obstruct the interplanetary transport of microorganisms in outer space.

The study of the influence of the conditions existing in outer space on terrestrial microorganisms is one of the important problems involved in space biology. Of course, this is only a part of the whole problem involved in the study of space which has been performed successfully in recent years by means of artificial Earth satellites and spacecraft.

In addition to the general theoretical interest that exists, the effect of extremal factors also has a practical significance, since the experimental confirmation of the death of microorganisms would render sterilization of spacecraft unnecessary.

In space, a number of physical factors operate which are missing on Earth but which can affect experimental systems.

The effects of low temperatures (Keilin, 1958) and ionizing radiation (Bak, Aleksander, 1963; Imshenetskiy, 1966) on microorganisms has been studied more or less and it is likely that these factors cannot be the cause of the death of microorganisms in space (Imshenetskiy, 1967). The greatest danger to microbes in space is posed by ultraviolet rays, but their lethal effect can be /107 offset by shielding the cells with cosmic dust, meteorites, etc. (Imshenetskiy,

1967). On the other hand, the effect of a high vacuum on microorganisms remains little studied. This is explained both by the novelty of the idea and the difficulty of obtaining a high vacuum close to that which exists in space (10^{-16}mm Hg).

Experimental Section

Description of Devices Used in the Experiments

At the present time, vacuum technology makes it possible to obtain pressures from 10^{3} to 10^{-14} mm Hg and below. It is impossible to use a single method to attain such a broad range of pressures, so that a combination of different types of pumps is employed as well as special absorbers and traps for vapors.

Let us examine one of these methods which was used in creating the high vacuum in a cryogenic high-vacuum system (Figure 1). The high-vacuum cryogenic system makes it possible to produce and maintain for a long period of time a pressure of 10^{-8}-10^{-10} mm Hg by means of nitrogen-hydrogen condensation pumps. The level of the pressure in the chamber depends on the gas and heat loads produced by objects located in the chamber. The operating principle of the condensation pump consists in the fact that the gases located in a volume cannot have a pressure greater than the pressure of saturated vapor at that temperature. Hence, their pressure in this volume is determined by the temperature of the coldest part under the condition that dynamic equilibrium is maintained throughout the volume. By reducing the temperature of this area, the gases can be caused to condense on it ("freeze out") and there will be a corresponding decrease in the total pressure owing to the reduction of the partial pressure of the condensed gases. If preliminary rarefaction has already been accomplished in the volume, i.e., if the total volume of gas is comparatively small, sufficiently deep cooling of even a small part can cause the significant drop in the pressure in the volume in question. The dimensions of the cooled area determine the rate of evacuation, and the temperature governs the ultimate vacuum which can be attained. To obtain a high vacuum in the chamber, a nitrogen condensation pump is used which has a temperature of -196°. This pump exhausts water vapor, added organic substances, CO_2, etc.

In addition, it is also a heat shield for the hydrogen condensation pump which serves to exhaust the nitrogen and the oxygen, protecting the latter against the thermal radiation from the walls of the chamber. Since the condensation pumps expel only the gases which condense on their surfaces, diffusion pumps /108
are used to expel noncondensed gases (for example, hydrogen or helium) which simultaneously serve for preliminary rarefaction. They are connected to the chamber by means of a nozzle. The latter contains the nitrogen trap for freezing out the oil vapors and connectors with a system of valves which make it possible to establish a connection between the chamber and the atmosphere or to interrupt exhaustion while the pumps are working, to perform exhaustion with mechanical pumps, shutting off the diffusion pumps, or to accomplish it with each of the diffusion pumps in sequence.

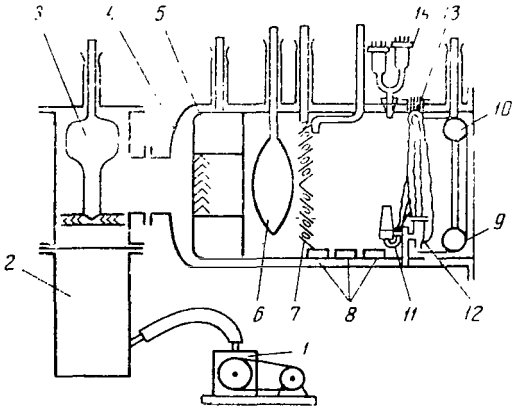

Figure 1. Diagram of Vacuum System. 1, Prevacuum pump, VN-2; 2, Diffusion pump N-5; 3, Nitrogen trap (trap cooled by liquid nitrogen); 4, Body of chamber; 5, Nitrogen screen; 6, Hydrogen pump (ergopump, cooled by liquid hydrogen); 7, Shutters cooled by vapors from liquid nitrogen; 8, Weighing bottles; 9, Lid of vacuum chamber; 10, Trap cooled by liquid nitrogen; 11 and 12, Manometric sensors LM-2 and IM-11 (MI-12); 13, Hermetically sealed intake; 14, Dual lamps LT-2 and LM-2.

Materials and Methods

Studies were performed with microorganisms belonging to various taxonomic groups (15 types of bacteria, 7 types of fungi, 7 types of yeast, 5 types of water plants). The taxonomic positions of these species will be described later.

All of the microorganisms were cultured on dense nutrient media -- meat-peptone agar, must-agar, Tamiya medium (Tamiya et al., 1953) after which aqueous /109
suspensions were prepared from the cells of the bacteria and the yeasts and from the spores of the fungi and bacteria. We applied

0.05 ml of an aqueous suspension of microorganisms to the surface of round filter paper, 10-12 mm in diameter. The filters were then placed in sterile Petri dishes, and the latter were then placed in a thermostat for 48 hours at 40° to dry the filters. The mycelium of the fungi, which did not contain spores, and the water plants were placed in the form of pieces of biomass on membrane filters (No. 3 filters) which were dried in a thermostat at 28° for 24 hours.

The samples obtained in this fashion were then transferred to glass weighing bottles with calcined and fiberglass-insulated potassium chloride. The weighing bottles were covered with caps and sealed with a layer of paraffin.

Prior to the experiment, the prepared filters were transferred to glass thin-walled sterile weighing bottles and placed in opened form on a nitrogen screen in a cryogenic high-vacuum chamber. The suspension of nonsporogenous bacteria was placed on the paper filters immediately prior to the placement of the samples in the high-vacuum chamber. During the experiments, the temperature of the bottoms of the weighing bottles was -23°. The duration of the experiment was 72 hours. During the experiment, the pressure was equal to 10^{-8}-10^{-10} mm Hg. Control samples of the filters were stored at room temperature in glass weighing bottles over potassium chloride. At the end of the experiments, the experimental and control filters, excluding the membrane filters with fungous mycelium and water plants, were placed in flasks containing 25 ml of sterile tap water and the filters were completely destroyed as a result of agitation of the flasks.

The culture material obtained in this fashion was used in amounts of 0.05 or 0.1 ml to inoculate Petri dishes with meat-peptone agar (MPA) or must-agar (MA). The Petri dishes were kept in a thermostat at 28° for 72 hrs, after which the number of colonies which had developed both in the experimental and control samples were counted.

Membrane filters (control and experimental) were transferred after the experiment together with the pieces of fungous mycelium and water plants to the surface of a nutrient medium (MA or Tamiya medium), after which the Petri

dishes containing MA were kept in the thermostat for 72 hours at 28°. The Petri dishes containing water plant samples were placed in the thermostat (28°) exposed to daylight and kept there for 5 to 22 days. The growth of the cultures on the membrane filters was used as an indication of the viability of the fungous mycelium and water plants.

Results of the Studies

In the first series of experiments, we studied the spores of bacteria belonging to the following species: *Bacillus lini, B. subtilis, B. mesentericus, B. mesentericus* var. *niger, B. mycoides, B. megaterium, B. simplex.* The data from these experiments are presented below (experimental conditions: /110
$P = 10^{-8}$-10^{-10} mm Hg, 72 hours).

Microorganism	Survival, %
Bacillus lini	57.6
B. subtilis	70.0
B. mesentericus	100.8; 84.2
B. mesentericus var. *niger*	75.6
B. mycoides	101.3; 97
B. megaterium	147
B. simplex	128.0

It is apparent from the results above that a high vacuum does not kill bacterial spores, since the smallest amount of intergrown spores was 57.6% (*B. lini*) and the maximum was 147% (*B. megaterium*) relative to the control, which was assumed to be 100%. The results obtained indicate that for the spores of certain bacteria, being situated under conditions of a high vacuum and low temperature was more favorable than keeping them at room temperature and ordinary atmospheric pressure.

The second series of experiments was performed with the following non-sporogenous bacteria: *Micrococcus luteus, Micr. aurantiacus, Sarcina flava, Pseudomonas pyocyanea, P. fluorescens, Escherichia coli, Serratia marcescens* and *Vibrio metchnikovii.*

The results of these experiments are presented below.

Microorganism	Survival, %
Micrococcus luteus	55.5
M. aurantiacus	51.7
Sarcina flava	68.5
Pseudomonas pyocyanea	0.34
P. fluorescens	0
Escherichia coli	4.7
Serratia marcescens	0
Vibrio metchnikovii	0

The data on survival of nonsporogenous bacteria under a high vacuum indicate that these organisms react differently to the action of a high vacuum. Thus, in three species (*P. fluorescens, Serratia marcescens, Vibrio metchnikovii*) all of the cells died in a high vacuum. In the case of two other species (*P. pyocyanea, E. coli*) the principal mass of cells perished and only in the coccic forms (*Micr. luteus, M. aurantiacus, Sarcina flava*) did the number of dead cells amount to less than 50%.

The following series of tests was performed with conidia, spores and /111
mycelium of fungi which did not contain spores.

Microorganism	Survival, %
Chaetomium globosum (mycelium)	Growth
Coriolus pubescens (mycelium)	"
Fomes fomentarius (mycelium)	"
Aspergillus oryzae	130.8
Asp. terreus	123.9
Penicillium wortmanni	31.5
Trichothecium roseum	4.4
Actinomyces globisporus (streptomycini)	128

These results indicate that in the case of *Aspergillus oryzae, A. terreus* and *Actinomyces globisporus (streptomycini)*, more viable spores remain under vacuum conditions than when the organisms are stored under laboratory conditions. The conidia of *Penicillium wortmanni* and *Trichothecium roseum* are less resistant to vacuum and the percentage of their survival is 31.5 and 4.4, respectively. In addition, the vegetative mycelium of fungi which do not contain spores also possesses considerable resistance to the effect of a high vacuum. When mycelia of *Chaetomium globosum, Fomes fomentarius* and *Coriolus pubescens* are exposed to a high vacuum, the mycelia of all three forms retain their viability completely.

In a fourth series of tests, we investigated the resistance of yeasts (*Saccharomyces vini, Zygosaccharomyces vini, Torulopsis aeria, Candida tropicalis* and *Rhodotorula rubra* to the action of a high vacuum).

Microorganism	Survival, %
Saccharomyces vini	25.9; 18.4
Zygosaccharomyces vini	0.77; 1.33
Torulopsis aeria	0; 0; 0.64
Candida tropicalis	33.54; 33.9
Rhodotorula rubra	10; 7

Only two forms -- *Saccharomyces vini* and *Candida tropicalis* -- were relatively resistant to the action of this factor. The other three forms -- *Zygosaccharomyces vini, Torulopsis aeria* and *Rhodotorula rubra* -- died out almost completely when exposed to the high vacuum.

In the last series of experiments, we studied the relationship of water plants (*Chlorella vulgaris, Scenedesmus acuminatus, Lyngbya aestuarii, Mastigocladus laminosus, Amorphonostoc puncliforme*) to vacuum. It was found /112 that after 72 hours spent in a high vacuum, all of these forms of water plants remained alive. However, microscopic examination of the cells and recording of the time required for restoration of pigment in the cells revealed several peculiarities. Thus, cells of *C. Vulgaris* and *Lyng. aestuarii* lost their green coloring immediately after transfer of the samples from the high vacuum to the solid nutrient medium. However, microscopic examination of the colorless mass of cells of *C. vulgaris* revealed individual dividing cells. Restoration of green coloration in the cells was observed on the fifth through seventh days. After this time, there were individual green spots which appeared in the colorless mass lying on the membrane filter. Restoration of green pigment in the culture of *L. aestuarii* took place only after three to four weeks. Control samples of these two forms of water plants did not lose their green coloring. The loss of green pigment was also observed in a culture of *S. acuminatus* both in the experimental and control batches. In addition, those cells which had been subjected to the action of a high vacuum showed septicization of the chloroplasts. Biosynthesis of the pigments took place first in the controls and then in the experimental cells. Similar

changes were not observed when other species of water plants were exposed to a high vacuum (*Mastigocladus laminosus* and *Amorphonostoc punctiforme*).

Study of the Activity of Dehydrogenase

In studying dehydrogenase, we used the formazane reaction, in which the hydrogen donor was 0.5 M sodium succinate or 0.5 M ethyl alcohol, while the hydrogen acceptor was 0.5 M solution of neotetrazolium chloride. The studies were performed with a culture of *Azotobacter vinelandii*, which was grown in a solid nutrient medium which did not contain nitrogen (Frobisher, 1965). Following 72 hours of culturing at 28°, phosphate buffer was added to the Petri dishes (pH = 6.5-7.2) and the cells were removed with the aid of a magnetic stirrer. The suspension of cells was washed three times in phosphate buffer with subsequent centrifuging (10,000 rpm, 15 minutes). The biomass of cells obtained in this fashion was applied in a thin layer to the bottom of Petri dishes and the latter were dried in a thermostat at 28° for 24 hours, after which the Petri dishes were placed on a nitrogen screen in a high vacuum chamber. The cells were under high vacuum for 72 hours. Then the cells were resuspended in a new batch of phosphate buffer until an optical density of 0.35 was obtained (in the first experiment) and until 1.05 was obtained (in the second experiment), using the FEK-N-57 and a No. 9 blue filter.

The results of these experiments are listed below. /113

0.5 M sodium succinate		0.5 M ethyl alcohol	
Suspension		Formazane	
0.35		0.35	0.24 0.119
0.35		0.35	0.33 0.08
Suspension		Formazane	
1.05		1.05	0.26 0.12
1.06		1.06	0.65 0.24

In comparing the optical densities of formazane in the two experiments, it becomes evident that there was no significant difference in the activity of the succinodehydrogenase and the alcoholodehydrogenase in the experiment and controls. The slight change in optical density of the formazane may be due to errors in the method.

Influence of High Vacuum on the Oxidation Processes of *Sarcina flava* and *Bacillus simplex*

Studies were performed with spores of *Bacillus simplex* and cells of *Sarcina flava*. As was established earlier, these cultures were distinguished by their sensitivity to the effect of high vacuum. The intensity of the oxidation processes in these microorganisms was determined polarographically on the basis of the rate of utilization of oxygen from the solution (Lysenko, 1969).

As the substrates, we used ethyl alcohol and glucose. The experiments were performed with four-day cultures grown in Petri dishes with a meat-peptone agar. In the culture of *B. simplex*, the number of spores formed was nearly 100%. The cells of *S. flava* and the spores of *B. simplex* were rinsed with nutrient medium containing phosphate buffer, centrifuged and prepared for exposure to a high vacuum by a method similar to that used for determining dehydrogenase. The duration of the experiments under vacuum was 72 hours. The temperature of the bottom of the Petri dishes during the experiments varied from -25 to -35°. The control Petri dishes containing microorganisms were stored at 20-25° and atmospheric pressure. The cells, both those that were subjected to the action of a high vacuum (10^{-8}-10^{-10} mm Hg) and the controls were then resuspended in a new batch of phosphate buffer (pH = 6.8-7.2) until an optical density of 0.50 was reached (FEK-N-57, No. 9 blue filter). The oxidation processes in *S. flava* were studied on the second day of resuspension, while in the case of *B. simplex* this was done on the third day. The studies of the suspensions were conducted at 35°.

Results Obtained

/114

The data on the effect of a high vacuum on the activity of the oxidation processes in *S. flava* are shown in Figures 2 and 3. The curves plotted in the diagrams illustrate the intensity of absorption of oxygen by a culture of *S. flava* with the addition of two different substrates to the suspension of cells (0.3 M ethyl alcohol and 0.3 M glucose). In both cases, following the action of a high vacuum, we found a significant reduction in the absorption of oxygen by the sarcina cells. A decrease in the quantity of oxygen absorbed by

these suspensions was also detected in cell suspensions which did not contain a specific substrate, i.e., with endogenic respiration.

A reduction of the intensity of oxidation of substrates in experimental suspensions may apparently be explained by inhibition or inactivation of the enzyme systems in the case of cells of *S. flava*, caused by a high vacuum.

In this connection, it was necessary to study the oxidation ability of forms more resistant to the action of a high vacuum. It was shown in the preceding chapter that spores of *B. simplex* which had been placed on the surface of filter paper and placed under high vacuum of 10^{-8}-10^{-10} mm Hg for 72 hours retained their viability completely. Figures 4 and 5 show data on the absorption of oxygen by suspensions of spores of *B. simplex* in a medium containing 0.15 M ethyl alcohol and 0.15 M glucose. The results obtained indicate an insignificant difference in the intensity of absorption of oxygen in the experimentals and the controls during the first 10 minutes. The suspension of cells in ethyl alcohol showed a much more rapid utilization of oxygen by the cells, subjected to the action of a high vacuum, in comparison to the controls (Figure 4). On the other hand, the suspension of spores with glucose revealed a more intensive bonding of the oxygen in the controls (Figure 5). The amount of oxygen absorbed during the experiment by the suspension of spores in phosphate buffer without substrate was approximately the same in both cases (Figures 4 and 5).

Evaluation of the Results

The study of the resistance of a number of microorganisms belonging to various systematic groups to a high vacuum revealed that the conidia of the genus *Aspergillus*, the spores of actinomycetes as well as some forms of bacteria (*Bacillus simple, B. megaterium*) possess a high level of resistance to this factor. Obviously these microorganisms retain their viability in a vacuum better at certain periods of exposure and low temperature than when kept in laboratory conditions.

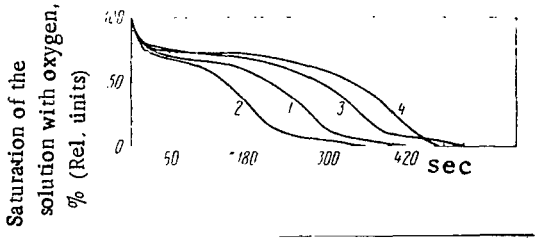

Figure 2. Absorption of Oxygen by Cells of *Sarcina flava*, Subjected to the Action of a High Vacuum.
1, 5 ml of cell suspension + 5 ml of 0.3 M ethyl alcohol (E);
2, 5 ml of cell suspension + 5 ml of 0.3 M ethyl alcohol (C);
3, 5 ml of cell suspension + 5 ml of phosphate buffer (E);
4, 5 ml of cell suspension + 5 ml of phosphate buffer (C).

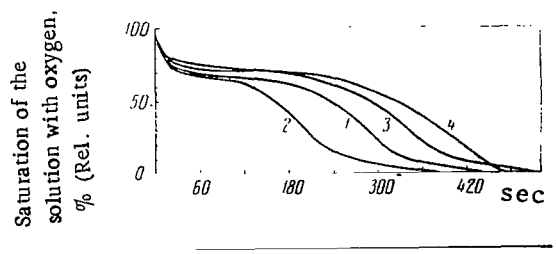

Figure 3. Absorption of Oxygen by Cells of *Sarcina flava*, Subjected to the Action of a High Vacuum.
1, 5 ml of cell suspension + 5 ml of 0.3 M glucose (E); 2, 5 ml of cell suspension + 5 ml of 0.3 M glucose (C); 3, 5 ml of cell suspension + 5 ml of phosphate buffer (E); 4, 5 ml of cell suspension + 5 ml of phosphate buffer (C).

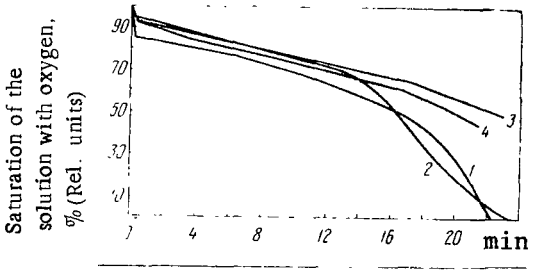

Figure 4. Absorption of Oxygen by Spores of *Bacillus simplex*, Subjected to the Action of a High Vacuum.
1, 5 ml of spore suspension + 5 ml of 0.3 M ethyl alcohol (E); 2, 5 ml of spore suspension + 5 ml of 0.3 M ethyl alcohol (C); 3, 5 ml of spore suspension + 5 ml of phosphate buffer (E); 4, 5 ml of spore suspension + 5 ml of phosphate buffer (C).

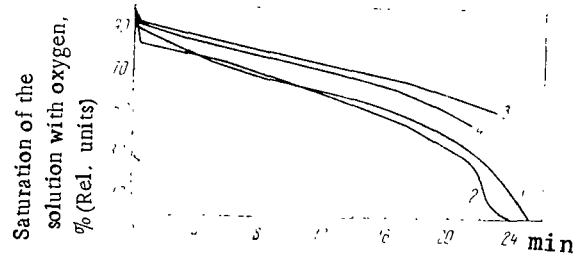

Figure 5. Utilization of Oxygen by Spores of *Bacillus Simplex*, Subjected to the Action of a High Vacuum.
1, 5 ml of spore suspension + 5 ml of 0.3 M glucose (E); 2, 5 ml of spore suspension + 5 ml of 0.3 M glucose (C); 3, 5 ml of spore suspension + 5 ml phosphate buffer (E); 4, 5 ml of spore spension + 5 ml of phosphate buffer (C).
E = Experimental, C = Control.

As far as the effects of a vacuum on nonsporogenous bacteria, yeasts, conidia of *Penicillium wortmanni* and spores of the fungus *Trichothecium roseum* are concerned, all of these turned out to be very sensitive to the effect of a high vacuum. The only exceptions were *Micrococcus luteus*, *M. aurantiacus* and *Sarcina flava*, which could be compared in terms of their resistance to such sporogenous forms as *Bacillus lini*, *B. subtilis* and *B. mesentericus* var. *niger*. It is still difficult to speak of a mechanism of resistance of microorganisms to a high vacuum. A few papers by Soviet and foreign authors have been devoted only to confirming the resistance of microorganisms to a high vacuum; even then the investigators obtained contradictory results. Thus, according to the data of Portner et al., (1961), spores of *Bacillus subtilis*, conidia of *Aspergillus fumigatus* as well as cells of *Mycobacterium smegmatis* did not die in a vacuum ($3.6 \cdot 10^{-10}$ mm Hg) in the course of five days. On the other hand, according to the data of Brueschke et al. (1961), spores of *B. subtilis*, conidia of the fungi *A. niger*, *A. terreus* and *P. citrinum* perished completely after 30 days exposure under vacuum equal to $1 \cdot 10^{-6}$-$6 \cdot 10^{-9}$ mm Hg. Obviously the question of the periods of retention of viability in the case of microorganisms kept under conditions of high vacuum requires more detailed studies. It should be pointed out, however, that the important changes, for example mechanical destruction of cells or changes in the exterior appearance of the colonies under the influence of a high vacuum were not observed. /116

In clarifying the mechanism of the action of a high vacuum on a cell, it is interesting to study certain functional characteristics of the microorganisms. In this connection, studies were also performed to clarify the dehydrogenase activity of *Azotobacter vinelandii* and the oxidation functions in *B. simplex* and *S. flava* following their exposure to a high vacuum. It was found that succinodehydrogenase and alcoholodehydrogenase activity in the experimental and control cells of *A. vinelandii* were at approximately the same level. The intensity of the oxidation functions in microorganisms exposed to the action of a high vacuum is also quite high. Thus, in the case of spores of *B. simplex* we did not observe any significant difference in the rate of oxidation of ethyl alcohol and glucose either in the controls or in the

experimentals. A slight decrease in oxidation capacity was observed in experimental samples of nonsporogenous cultures of *S. flava*: after 72 hour exposure to a high vacuum, about 31% of the cells died. All of this indicates that microorganisms not only survive but also retain their physiological activity following 72 hours exposure to a high vacuum. Consequently, if we consider the high resistance of certain microorganisms to the effect of ionizing radiation, temperature, ultraviolet rays as well as the possible /117
shielding of microbes in space against ultraviolet radiation from cosmic dust, meteorites and spacecraft, it becomes clear that the "transport" and "import" of microorganisms through outer space is completely practical. The only possibility of preventing transfer of microorganisms to space and to other planets can be accomplished by careful sterilization of spacecraft.

The authors would like to express their profound gratitude to the Director of the Physical-Technical Institute of Low Temperatures of the Academy of Sciences of the Ukrainian SSR, Corresponding Member of the Academy of Sciences of the Ukrainian SSR, B. I. Verkin, for making it possible for us to carry out our experiments in a high vacuum.

Conclusions

The data which we obtained make it possible to divide the microorganisms which were studied according to their resistance to the action of a high vacuum into the following groups.

1. Highly resistant microorganisms with a survival rate of 100% or more (*Bacillus mesentericus, B. mycoides, B. megaterium, B. simples, Aspergillus oryzae, A. terreus* and *Actinomyces globisporus (streptomycini)*.

2. Resistant forms, whose survival rate was 76 to 52 (*Bacillus lini, B. subtilis, B. mesentericus* var. *niger, Micrococcus luteus, M. aurantiacus* and *Sarcina flava*).

3. Partly resistant microbes whose survival rate was 22 to 34% (*Saccharomyces vini, Candida tropicalis*).

4. Nonresistant microorganisms which perished completely in a vacuum or whose number of surviving cells was equal to several percent (*Pseudomonas*

fluorescens, *Serratia marcescens*, *Vibrio metchnikovii*, *Zygosaccharomyces vini*, *Torulopsis aeria*, *Rhodetorula rubra*, *Trichothecium roseum* and *E. coli*).

At the same time, mention should be made of the survival of mycelium and water plants following their exposure to a high vacuum as well as the retention of oxidation functions by *B. simplex* and *Sarcina flava*.

5. A high vacuum (10^{-8}-10^{-10} mm Hg) cannot serve as a barrier to the transfer of microorganisms through space to other planets so that sterilization of spacecraft is necessary.

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BEHAVIOR OF CERTAIN SOIL MICROORGANISMS IN THE "ARTIFICIAL MARS" CHAMBER

L. A. Kuzyurina and V. M. Yakshina

ABSTRACT: The possibility that certain soil microorganisms could grow under conditions of maximum hygroscopic moisture is discussed within the parameters of the Martian climate (temperature from -60 to +26°, atmospheric pressure equal to 7 mm Hg, gas composition equal to 80% carbon dioxide plus 20% nitrogen). Microorganisms isolated from the soil of the Pamir, the Karakum Desert, and soil from Dixon Island, were found to be resistant to varying degrees to living conditions in the "artificial Mars chamber". The oligonitrophilous mycococci withstand these conditions best of all. This microorganism multiplies. The number of cells on the 14th day has increased by 7.6 times or more.

So far, before biological stations have been sent to other planets so that they can provide a clear answer to the question of whether or not there is life on these planets, the study of the effect of extremal factors on terrestrial organisms has been a topic of considerable interest. These studies make it possible to determine the ability of terrestrial organisms to develop under conditions that exist on other planets, especially Mars.

In an artificial climate chamber, pathogenic spore and nonspore forms of microorganisms are placed, the effect of Martian conditions on the physiology and virulence, ability to develop spores and multiply are studied in representatives of various physiological groups of microorganisms under artificial conditions resembling those on Mars (Davis et al., 1959; Hawrylewicz et al., 1962; Roberts, 1963; Hawrylewicz et al., 1964, 1966; Hagen et al., 1967). Roberts (1963) showed that *Rhodospirillum rubrum* is capable of photoassimilation of carbon dioxide and fixation of nitrogen under conditions of an "artificial Mars". The parameters of the conditions in these experiments are as follows: a mixture of gases consisting of carbon dioxide (2.21%) and nitrogen (93.54%); the moisture of the red lava was about 0.5% for 1 gram while the daytime temperature was 22-25° and that at night was -25°.

The soil microorganisms that were most frequently tested under conditions of the artificial climate were *Bacillus cereus* and *Bacillus subtilis*, as some

of the most widespread sporogenous forms on the Earth. We studied their survival, multiplication and growth of their spores.

In the opinion of the majority of authors, the principal factor which limits the survival of terrestrial microorganisms under Martian conditions is the low humidity. It should be pointed out that the conditions in the /119
"artificial Mars" chambers were different. This is based on information regarding the conditions that actually exist on Mars as well as on the individual theories of the authors.

It is possible that there is a certain analogy between the xerophytic microorganisms and the frost-resistant seeds of plants which can withstand dehydration readily. Proceeding on the ecological principle, one should use for experiments in the "artificial Mars" chamber microorganisms that are found in the soil of deserts, high mountains, and in Arctic and Antarctic regions, where the conditions of life are highly similar to those on Mars.

In this article, we shall describe the results of a study of the survival of xerophytic soil bacteria in an "artificial Mars" chamber. The soil bacteria were separated by us from high mountain Pamir serozems, dehydrated desert soil from the Karakum Desert and soil from Dixon Island. In addition, their species membership was also established.

Experimental Section

Method

Experiments were performed in short test tubes with serrated edges to allow free passage of gases. These test tubes were covered with broad glass lids, and 1 gram of limonite melkozem was added to each (particle diameter equals 0.25 mm) as well as 2% garden soil. The mixture of limonite with the soil was sterilized, dried and moistened with a suspension of bacterial cells so that the mixture had a maximum hygroscopic moisture of 3.8%. To determine the humidity at the end of the experiment, we added water to 5 grams of a mixture of limonite with soil contained in weighing bottles such that the maximum hygroscopic moisture was achieved and the weighing bottles were placed in the "artificial Mars" chamber in the same fashion as the experimental test

tubes. The description of the artificial climate chamber has been given in a paper by Zhukova and Kondrat'yev (1965).

A suspension of bacterial cells (density 0.04 in cell 3 with red light filter, according to the FEK-56 nephelometer) was prepared from 1-day agar cultures and placed in the test tubes with the aid of a micropipette.

The experiments were performed under harsh extremal conditions with an extremely high content of carbon dioxide in the gas mixture, a sharp variation in temperature, low pressure and maximum hygroscopic humidity of the mixture of the limonite and soil.

The parameters of the conditions in the "artificial Mars" chamber were as follows. The pressure was 7 mm Hg. The mixture of gases (steady flow) was /120 made up of 80% carbon dioxide and 20% nitrogen and passed through a saturated solution of potassium sulfate to maintain maximum hygroscopic moisture.[1] For this purpose, a weighing bottle containing a saturated solution of potassium sulfate was placed in the chamber, and the contents were replaced as they evaporated. The temperature varied between -60 and +30 according to the diurnal curve on the equator of Mars (Vokuler, 1956; Moroz, 1965; Kliore et al., 1965; Sinton, Strong, 1960). The positive temperature was maintained for 8 hours a day. The mixture of limonite with 2% garden soil was not irradiated with ultraviolet rays, since even very thin layers of soil completely absorbed the rays.

The studies were performed with the following bacteria: *Bacillus zoogleicus* and *Micrococcus* sp. (obtained from Pamir serozems), *Bacillus aegypticus* (taken from soil in the Karakum Desert), and *Mycococcus oligonitrophilus* (from soil collected on Dixon Island).

The results of the experiments are shown in Figure 1. It follows from these data that not all microorganisms behave in the same fashion under the conditions of the "artificial Mars" chamber. After 14 days, 4.6% of the cells

[1] The gas mixture was prepared according to one of the three possible mixtures as determined by "Mariner-4" (Kliore et al., 1965).

of *B. zoogleicus* remained alive in the chamber. In the case of *Micrococcus* sp. under the same conditions, 7.3% of the cells survived while *Mycococcus oligonitrophilus* multiplied and the number of cells increased during this period in comparison to original by 7.6 times. A culture of *B. aegypticus* turned out to be resistant to Martian conditions. The number of cells at the end of the experiment was the same. /121

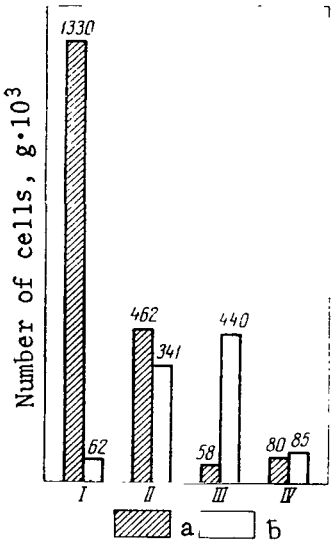

Figure 1. Influence of Conditions Existing in the "Artificial Mars" Chamber on Soil Microorganisms: Temperature from +25 to -60°. Gas composition: CO_2 -- 80%, N_2 -- 20%. Pressure equals 7 mm Hg. The limonite had a maximum hygroscopic moisture of 3.8%. The duration of the experiment was 14 days. a, Original number of cells; b, Number of cells after 14 days; I, *B. zoogleicus;* II, *Micrococcus* sp.; III, *Mycococcus oligonitrophilus;* IV, *B. aegypticus.*

Hence, the oligonitrophilous mycococcus isolated from the soil from Dixon Island turned out to be a form which was most adapted to extremal conditions of existence.

In a second group of experiments that were performed with the same culture under conditions of an "artificial Mars", but with a moisture equal to 4%, the ability of *Mycococcus oligonitrophilus* to multiply was retained. The number of cells of this microorganism on the 14th day of being kept in the "artificial Mars" chamber increased by a factor of 23, from $30 \cdot 10^3$ to $79 \cdot 10^4$ per gram of "soil".

A second series of experiments which lasted 14 days as well differed from the preceding in the fact that the temperature of these tests did not vary but remained constant (28°).

The data from the experiments are presented in Figure 2. They support the results that were obtained earlier. Bacteria will not

die under these conditions. The number of cells in the experiment with sporogoneous bacilli finally turned out the same as in the preceding series of experiments; the oligonitrophilous mycococci multiplied in limonite.

We then performed observations involving the same culture and it was found that *Mycococcus oligonitrophilus* will survive under conditions of the "artificial Mars" chamber even a lower soil moisture, from 0.2 to 1.8%. On the 14th day we found as many as 10^3 cells per gram of limonite and soil (Figure 2).

Seeding this inoculation material, diluted with water and stored for two days in a refrigerator at 5° always produced growth. However, the number of cells of *Mycococcus oligonitrophilus* decreased by a factor of 10 in comparison with the initial amount. In this connection, we performed tests of the effect of the moisture of the limonite containing 2% garden soil on growth and multiplication of *Mycococcus oligonitrophilus*, with the following method being used. Short test tubes with serrated edges (for improved passage of air and moisture) were covered loosely with glass caps and placed in desiccators of uniform size, on the bottom of which water (control) or a 10% solution of /122
sulphuric acid had been poured.

To create still lower moisture of the limonite, 50 or 96% sulphuric acid was added to the desiccators. To each experimental test tube, we added 1.0 g of sterile limonite and soil, brought to constant weight and 0.04 ml of an aqueous suspension of a 1-day culture of *Mycococcus oligonitrophilus*. This culture was grown on a meat-peptone agar at 30° and was used to prepare an aqueous suspension of cells with a density (according to the FEK-56) of 0.04 D (with a red light filter, cell No. 3). Addition of this amount of suspension of mycococci caused development of maximum hygroscopic moisture in the limonite.

The test tubes were placed in desiccators for 36 days. In addition, in order to determine the moisture of the limonite at the end of the experiment, weighing bottles containing 5.0 g limonite brought up to constant weight were installed with them, with addition of 0.2 ml of tap water to produce maximum hygroscopic moisture, i.e.,the same moisture as in the experimental test tubes.

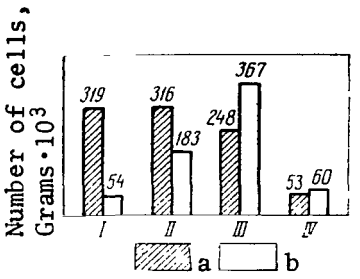

Figure 2. Influence of Conditions Existing in the "Artificial Mars" Chamber at a Constant Temperature of +28° (Temperature Control) on Soil Microorganisms: Gas composition: CO_2 -- 80%, N_2 -- 20%. Pressure equals 7 mm Hg. The limonite had 3.8% moisture (maximum hygroscopic). The experiments lasted 14 days. a, Original number of cells; b, Number of cells after 14 days; I, *B. zoogleicus;* II, *Micrococcus* sp.; III, *Mycococcus oligonitrophilus;* IV, *B. aegypticus*.

Counting of the number of microorganisms was performed with the aid of inoculation on Petri dishes with meat-peptone agar with subsequent counting of the number of colonies which developed in two days at 30°.

From the results of the tests that are listed below it is apparent that under conditions of ordinary pressure of a terrestrial atmospheric composition and at room temperature, oligonitrophilous micrococci are able to multiply at a substrate moisture level equal to 2.5% (i.e., below the maximum hygroscopic moisture of limonite, equal to 3.8%), while the number of cells increases by a factor of two in comparison to the original number in the course of 36 days. With a limonite moisture equal to 0.4 to 0.5%, the number of cells of the mycococcus in it decreases by a factor of 10, but they do not die completely. This indicates their xerophytic nature.

In the Desiccator in the Presence of:	Average Moisture of Limonite in 36 Days, %*	Average Number** of cells of Mycococcus in 1 g of Limonite after 36 days
Water	2.5	$20.2 \cdot 10^5$
10% H_2SO_4	1.2	$63 \cdot 10^4$
50% H_2SO_4	0.5	$10.8 \cdot 10^3$
95% H_2SO_4	0.4	$31.0 \cdot 10^3$

* Original moisture -- 3.8%
** Average original amount of mycococcus -- $66.2 \cdot 10^4$ per gram of limonite.

The fourth series of experiments which lasted seven days was performed in the "artificial Mars" chamber with sporogenous bacillus isolated from soil collected in the Karakum Desert. It was found that *Bacillus cylindrosporus* is well suited for a dry climate, temperature variations, gas mixture and reduced pressure. A certain number of cells were placed in a test tube containing limonite and garden soil (2%). This mixture was sterilized, dried and moistened to 3.8%. In the course of seven days, the control and experimental test tubes were kept in anaerostats over a saturated solution of potassium sulfate in order to maintain the maximum hygroscopic moisture of the limonite. /123

The experimental anaerostat was filled with a gas mixture composed of CO_2 -- 50%; N_2 -- 40% and Ar -- 10%, corresponding to our concepts of the Martian atmosphere on the basis of the data obtained with the aid of the "Mariner-4". Inside, a pressure equal to 10^{-2} atmospheric was produced. The daily variations in the temperature ranged from -60 to +28°. This was achieved by placing the anaerostat for 16 hours in a Dewar vessel with a mixture of alcohol and dry ice, and then moving the anaerostat for 8 hours to a thermostat where the temperature was kept at 28°. In the control anaerostat, there was no addition of a gas mixture. The air was not pumped out. The moisture of the limonite was the same. The temperature was kept constant at 28°. The amount of bacteria contained in the limonite at the beginning and end of the experiment was determined by means of cultures in Petri dishes with meat-peptone agar.

The data from these experiments indicate that in limonite with a maximum hygroscopic moisture there was development of sporogenous bacilli, *Bacillus cylindrosporus*. Seven days later, the number of cells in some of the experiments had increased by a factor of three. When comparing the results obtained in the experiments and the control tests it was found that the variations in temperature, composition of Martian atmosphere and vacuum had no effect on the growth of the bacteria being studied. This indicates that only low moisture of the substrate limits their growth.

Evaluation of the Results

It is convenient to assume that with maximum hygroscopic moisture of the soil, only certain actinomycetes and fungi can develop. Obviously, it will be necessary to make changes in this concept, since certain bacteria can also multiply in soil at the indicated moisture level. Experiments that have been performed indicate that the principal factor which limits the vital activity of terrestrial microorganisms under the conditions existing in the "artificial Mars" chamber is the moisture of the soil. Our experiments confirmed this.

In pointing out the extremely well developed adaptability to living conditions possessed by certain soil bacteria, both oligonitrophilous and saprogenic, Rakhno (1960) notes their ability to achieve maximum reproduction in winter in frozen soil, although they are not strict psychrophils. Obviously, microorganisms of this kind can withstand dehydration well.

The oligonitrophilous mycococcus isolated from the soil of Dixon Island /124
proved to be the most resistant and capable of reproduction of all the microorganisms exposed to the conditions in the "artificial Mars" chamber and to dehydration. This constitutes a certain degree of support for the author's opinion regarding oligonitrophilous forms. Probably the xerophytic and halotolerant forms of microorganisms will prove to be the ones most able to adapt to the extreme Martian climate conditions. The possibility cannot be excluded that the "osmophilic life forms" will be able to multiply under the conditions existing on Mars, where there is very little moisture.

It should be pointed out that all of the microorganisms which we studied that were isolated from desert and high altitude soils proved to be halotolerant, growing in a medium with 15% sodium chloride. Not a single microorganism mentioned in this work perished under the extremal Martian conditions and had adapted to them to a greater or lesser degree.

In conclusion, we would like to express our deep appreciation to A. A. Imshenetskiy for directing our work and for valuable comments.

Conclusions

1. Xerophytic microorganisms isolated from the soil collected in the Karakum Desert, the high altitude regions of the Pamir and from the soil on Dixon Island proved to be resistant to varying degrees to artificial Martian conditions.

2. The oligonitrophilous mycococcus *(Mycococcus oligonitrophilus)* multiplies under the conditions of the "artificial Mars" chamber with maximum hygroscopic moisture of the limonite (3.8%). The number of cells was found to increase by a factor of 7.6-23 by the end of 14 days.

3. After 14 days, 4.6% of the cells of *Bacillus zoogleicus* were found to survive in the "artificial Mars" chamber.

4. *Bacillus aegypticus* proved to be resistant to Martian conditions. The number of cells was not reduced at the end of the experiment.

5. *Bacillus cylindrosporus* develops in limonite with 3.8% moisture in the "artificial Mars" chamber, and the number of cells increases by a factor of three in seven days.

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POSSIBILITY OF THE SPREADING OF VIABLE GERMS IN OUTER SPACE

R. I. Fedorova

ABSTRACT: The possibility of germ survival and transport in outer space is discussed on the basis of present theories and experimental data, including also studies of material particle transport between celestial bodies by Arrhenius (1909, 1911), and studies by Oparin (1957), Imshenetskiy (1964) and Shklovskiy (1965) who came to the conclusion that among other unfavorable environmental factors UV radiation is most detrimental for the survival of germs and spores in outer space. UV radiation inactivation doses are given for a large group of germ strains. Recent experiments concerning survival of *Bacillus megaterium* spores exposed to UV radiation are described, showing that spores can survive bactericidal UV doses when shielded by a layer of tuff dust. Similar experiments with *Bacillus cereus* spores protected by chromium films are also described.

In these times, characterized by an intensive program involving the conquest of space -- the interest of scientists in the effect of factors in the space environment on biological objects has seen intensive growth. Detection of bactericidal properties of space factors would make it possible to solve the problem of the possibility of transporting viable germs through space, exchange of living organisms between planets, which has not only considerable theoretical importance but also no small amount of practical significance in conjunction with the necessity for sterilizing spacecraft.

The idea of the spread of microorganisms through space to other planets is not a new one. Thus, in 1865 Richter suggested that the seeds of life might have reached the Earth with particles that were torn away from heavenly bodies and 20 years later the German physiologist Helmholz proposed the idea of the possibility of collecting the spores of microorganisms together with meteorites. The hypothesis has been suggested by many individuals, but all of them belong to one of two schools: transport of germs by meteorites (the hypothesis of cosmozoism or lithopanspermy) and the transport of germs together with space dust as the result of the action of the pressure of light (the hypothesis of radiopanspermy).

The famous Swedish physicist and chemist Arrhenius (1909, 1911), proceeding on the basis of the pressure of light rays which was theoretically discovered by Maxwell and demonstrated in splendid experimental fashion as well as measured quantitatively by the Russian physicist Lebedev, studied the problem of the transport of particles of matter from one heavenly body to another in great detail. According to his theory, air currents flowing upward, /126 especially the strong ones which develop when volcanoes erupt, may carry very fine particles to altitudes as great as 100 km or more above the surface of the Earth. Electrical discharges in the upper layers of the atmosphere are capable of ejecting these particles of material from the Earth's atmosphere into interplanetary space, where the movement of the particles will take place under the influence of unilateral light pressure from the Sun's rays. Arrhenius calculated that spores of bacteria with a diameter of 200 to 150 millimicrons could reach the limits of our planetary system in 14 months.

A number of the objections of a philosophical nature have been raised against the theory of Arrhenius, involving the origin of life on Earth. However, the idea of the possibility of transfer of spores of microorganisms from planet to planet does not contradict the materialistic view of philosophy.

At the present time, the theory of panspermy has been reevaluated by many scientists in the light of modern astronomical data.

Lederberg (cited in Clemedson, 1964) feels that there is no natural mechanism for ejection of sporogenous particles from the gravitational field of planets of the Earth type or from any other rather large heavenly body which has an atmosphere.

Sagan (cited in Clemedson, 1964) concluded that the light pressure of the solar rays could only expel into outer space organisms measuring 0.2 to 0.6 micron in diameter. The pressure of the light from stars with smaller masses than the Sun would be insufficient to eject microorganisms into space. Only stars in the spectral interval from A0 to G5 could serve as sources for the spread of life in this fashion. However, Sagan emphasizes that the Earth received one microorganism from a stellar source during the first billion years of its history, if each of the 10^{11} known stars in our galaxy ejected

about one ton of microorganisms into interstellar space during this period of time.

According to the calculations of Anders (1961), each of the 10^{11} planets in our Solar System* would have to eject $6.0 \cdot 10^{18}$ microorganisms in order for one microorganisms to land on 1 m^2 of the lunar surface. Anders also points out that the possibility that the Moon received terrestrial microorganisms is greater than the possibility that it received them from other planets and therefore it may be possible to find forms of microorganisms there which have already died out on Earth.

Hence, at the present time scientists do not exclude the possibility of natural spread of life from planet to planet, although they feel that the probability of such a propagation is very small.

In order to have a general idea of the theory of panspermy, it was necessary to establish the possibility of retaining the viability of embryos during their long journey through space. Acting on the basis of the achievements of /127
modern natural science, the majority of famous scientists (Oparin, 1957; Ishmenetskiy, 1964; Shklovskiy, 1965) have concluded that if all the factors unfavorable to life which exist in outer space (low temperatures, close to absolute zero, absence of moisture, lack of oxygen, an ultrahigh vacuum on the order of 10^{-16} mm Hg, diverse forms of radiation, etc.), the principal obstacle to the retention of the spores of bacteria in a viable state is ultraviolet radiation. The principal source of ultraviolet radiation in the Solar System is the Sun. The density of the total flux of electromagnetic radiation from the Sun at its average distance from the Earth (one astronomical unit) equals $1.40 \cdot 10^6$ ergs/cm^2/sec or 0.140 W/cm^2 ± 2% (Jaffe, Rittenhouse, 1962). Approximately 1% of the total energy falls in the wave length region below 3,000 Å. This portion of the radiation is absorbed completely in the Earth's atmosphere. Since these waves are shorter than 2,800-2,900 Å, life on our planet has become accustomed to life under ultraviolet radiation with a wave length higher than 2,800 Å as a result of long evolution. It is possible that this is part of the

*Editor's Note: Fedorova's error. She obviously means the 10^{11} stars in our Galaxy.

reason why the greatest bactericidal effect is exhibited by the rays which are below this limit, in the range from 2,500-2,700 Å. The intensity of solar radiation in the orbit of Venus is 1.9 times greater than in the Earth's orbit, while in the orbit of Mars it is about 0.4 of the intensity of the dolar radiation in the Earth's orbit. Reflected solar light considerably increases the total intensity near the Earth and planets. In addition, ultraviolet radiation in the Earth itself and from the planets spreads into outer space.

The intensity of ultraviolet radiation at the 2,500-2,600 Å wave length in space at a distance of one astronomical unit is equal to $2 \cdot 10^3$ ergs/cm^2/sec (Jaffe, Rittenhouse, 1962). This means that if a spore is ejected from the Earth and moves in a straight line toward Mars at the speed of sunlight, it will reach Mars in an average of 1090 hours. During this time, the spore will receive a dose of ultraviolet radiation in the bactericidal range of the spectrum of $7.85 \cdot 10^9$ ergs/cm^2. The sensitivity of microorganisms to ultraviolet /129 light varies. In the experiments of Panasyuk (1966), the resistance of yeasts to ultraviolet light in the 2,650 Å range was 170 times higher than for bacteria. Solntseva (1967), studying high altitude strains of yeast-like organisms observed that within a single genus *(Rhodotorula)* the strains which were most resistant to ultraviolet light were 300 times more resistant than the most sensitive ones.

Wave length, Å	Relative Effectiveness, %	Wave length, Å	Relative Effectiveness, %
2200	25	2800	60
2300	40	2900	30
2400	63	3000	6
2500	91	3100	1.3
2537	100	3200	0.4
2575	100	3400	0.09
2680	99	3600	0.03
2700	87	4000	0.01

TABLE 1. SENSITIVITY OF VARIOUS MICROORGANISMS TO ULTRAVIOLET RADIATION (λ = 2537 and 2650 Å)

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Microorganism	Dose of UV·10^4 ergs/cm^2			Death of Cells, %
	Meyer & Seitz (1952)	Clemedson, (1964)	Samoylova (1967)	
Escherichia coli	1,55	3	1—2,5	90—100
Bacillus subtilis	—	7	6—7	90—100
Bac. subtilis (spores)	—	12	12	90—100
Bac megaterium	—	—	1,13	90—100
Bac. megaterium (spores)	—	—	2,73	90—100
Bac. pyocyaneum	4,4	—	—	90—100
Micrococcus sphaeroides	—	—	10	90—100
M. candicans	3,67	—	—	90—100
M. pyogenes aureus	—	—	6,0	90—100
M. lysodeikticus	—	—	27—50	90—100
M. radiodurans	—	—	80—160	90—100
Pseudomonas aeruginosa	—	—	1,8—3,6	90—100
Ps. fluorescens	—	—	3,0—3,5	90—100
Sarcina lutea	—	—	19,7	90—100
Salmonella typhimurium	—	—	1,9	90—100
Serratia marcescens	0,7	—	1,8—4,0	90—100
Staphylococcus albus	—	—	1,84—3,3	90—100
Staph. aureus	—	—	2,18—4,95	90—100
Streptococcus lactis	—	—	6,15	90—100
Str. ridans	—	—	2,0	90—100
Str. hemolyticus	—	—	2,16	90—100
Saccharomyces sp.	14,7	—	—	90—100
Sacch. cerevisiae	—	6	—	90—100
Saccharomyces (diploids)	—	—	30	90—100
Sacch. (haploids)	—	—	8,4	90—100
Sacch. vini (diploids)	—	—	30	90—100
Actinomyces sp.	—	—	4,0—8,0	90—100
Aspergillus niger	—	13	90—160	90—100
Asp. nidulans	—	—	54	90—100
Penicillium digitatum	—	44	—	90—100
Oospora lactis	—	5	—	90—100
Bacterium aertrycke	0,048	—	—	80
Bacillus megaterium sp.	2,9	—	—	80
Bac. megaterium (spores)	6,0	—	—	80
Saccharomyces turbidans	9,0	—	—	80
Sacch. cerevisiae	6,5	—	—	80
Staph. aureus	1,54	—	—	80

Translator's Note: Commas indicate decimal points.

Table 1 lists the data on the doses required for inactivation of 80-100% of the microorganisms (Meyer, Seitz, 1952; Clemedson, 1964; Samoylova, 1967). They vary within wide limits -- from 10^2 to 10^6 ergs/cm^2. Thus, the dose of ultraviolet radiation which a spore will receive on its flight from Earth to Mars will be so much higher than the bactericidal dose for the most resistant terrestrial microorganisms that there will be no question of its death during the trip.

But this position is valid for unprotected spores.

According to the data of Zhukova and Kozlova (1966), a shield composed of a single layer of cells of *Rhodotorula colostri* (cell diameter equals 1.9 microns) reduces the bactericidal effect of ultraviolet rays by a least a factor of four. This shielding of the cells by superjacent individuals in growing colonies was mentioned in 1901 by Tomashevskiy. In the experiments of Vashkov and Serebryakova (1960) with spores of Anthracoid and potato bacillus, placed on slides, considerable differences in the resistance of the spores to ultraviolet light were observed depending on whether the surface of the slide was infused with water or a bouillon suspension. Spores from the bouillon culture, in the dried state, proved to be 20 times more resistant to ultraviolet rays than the spores from the aqueous suspension. It is logical to assume that in this case a protective effect was produced by the layers of organic substance that formed on the cells due to the bouillon suspension following its drying. Obviously, similar phenomena are observed under natural conditions. A necessary condition for the growth of all life on Earth is the presence of water, and it is therefore difficult to imagine that spores would enter the air from various habitats of microorganisms which would not be carrying with them adsorbed particles of solutions from the ambient medium. We also know that microorganisms rapidly perish on smooth surfaces and in dust free air from ultraviolet radiation, faster than they do on rough surfaces and in dusty air (Kichenko et al., 1950; Ebert, Weil, 1955).

Imshenetskiy (1964) suggested that a spore could be protected against ultraviolet radiation in space by projecting structural elements of the dust mote on which it was located.

The literature contains practically no data on the depth of penetration /130
(depending on relaxation length) of individual rays in the ultraviolet spectrum for diverse substances, minerals, possible carriers of germs of life in space. We know that the relaxation length of ultraviolet rays is largely dependent upon the properties of the substance. In this connection, it was interesting to determine the protective possibilities of dust of both cosmic and terrestrial origin. The existence of dust particles in the upper layers of the atmosphere is supported by the results of collecting micrometeorites using rockets. Thus, out of all the particles detected at an altitude of 88-168 km, only 20% turned out to be solid, nearly spherical, resembling meteorites; the remaining 80% had a completely terrestrial shape with an open structure (Mikrometeority [Micrometeorites], 1963). In our experiments with dust particles from tuff, we obtained significant results.

Dry spores of *Bacillus megaterium* (in % of the control) were pulverized beneath a glass hood by means of a fan, which was replaced after a certain period of time following the beginning of pulverization of the spores by open sterile Petri dishes. Then a layer of dust from tuff was applied to a portion of the "seeded" dishes. The spores which were placed in a thin layer in the dishes and the spores which were covered with dust from above were subjected to the action of ultraviolet rays (BUV-30P bulb) for 1.5 hours. Then the controls (nonirradiated) and the experimental dishes were filled with meat-peptone agar and placed in a thermostat. The number of colonies which developed, as a percentage of the original number, served as an indicator of the protective effect of dust for spores irradiated with ultraviolet light.

No.of Exper.	Spores without dust	Spores mixed with dust	No of exper.	Spores without dust	Spores mixed with dust
1	1	100	5	5.3	100
2	0	64.5	6	0	63
3	2.3	83	7	5	75
4	1	100	8	0.7	25

Although the thickness of the dust layer could not be standardized from one experiment to the next, these tests did show that with a certain screen

thickness made of bits of tuff dust the spores can retain their viability under unconditionally bactericidal doses of ultraviolet light.

In addition, retention of viability was established for a considerable number of spores beneath rust (formed after placing a suspension of spores of *B. megaterium* on a sheet of ordinary steel) when exposed to irradiation by ultraviolet light for 1.5, 3 and 4.5 hours (doses: $4.2 \cdot 10^8$; $8.4 \cdot 10^8$ and $1.26 \cdot 10^9$ ergs/cm^2).

TABLE 2. EXPERIMENTS WITH SHEETS OF ORDINARY STEEL COATED WITH A LAYER OF RUST /131

Variant	Repetition	Number of Germinated Spores on Sheet: Nominal	% of Cont.	Nominal	% of Control
		Experiment I		Experiment II	
Control (without irradiation)	1	400 060	—	220 000	—
	2	200 120	—	200 580	—
	3	240 165	—	240 700	—
	4	200 097	—	240 105	—
Irradiation for 1.5 hrs, dose = $4.2 \cdot 10^8$ ergs/cm^2	1	100 140	38,4	64 130	28.4
	2	240 140	92,3	40 570	18
	3	240 150	92,3	45 700	20,2
	4	100 000	38,4	–	—
Irradiation for 3 hrs, dose = $8.4 \cdot 10^8$ ergs/cm^2	1	100 200	38,5	25 570	11,3
	2	100 080	38,4	126 100	55,9
	3	100 140	38,4	68 080	30,2
	4	100 047	38,4	—	—
Irradiation for 4.5 hrs, dose = $1.26 \cdot 10^9$ ergs/cm^2	1	100 075	38,4	10 370	4,6
	2	240 285	92,4	24 160	10,7
	3	240 110	92,3	—	—
	4	100 170	38,4	20 430	9,06

NOTE. In experiment I, the average number of spores, equal to 260110, was assumed to be 100%; in experiment II, the average number of spores was equal to 225350 and was assumed to be 100%.

Translator's Note: Commas indicate decimal points.

The percentage of germinated spores relative to the controls (nonirradiated sheets made of the same material, with spores) varied depending on the thickness and the area of the layer of rust which formed. With good covering, the survival rate reached 92%, while on sheets made of stainless steel the survival rate for spores of *B. megaterium* was only a thousandth of a percent (Tables 2 and 3). In these experiments, the spores of *B. megaterium* that

were under the protective screen of rust retained their viability at doses of ultraviolet light close to those which (according to the above calculations) may be received by a particle covering the distance from Earth to Mars (approximately equal to $7.85 \cdot 10^9$ ergs/cm^2).

Cosmic dust is composed of particles of extraterrestrial origin which, thanks to their small size, do not undergo considerable heating when they strike the Earth's atmosphere and reach the Earth in an unchanged form. They are usually referred to as micrometeorites. It is considered that space dust is of cometary origin and represents fragments with a density from 3 to 3.5 g/cm^3 or 7-8 g/cm^3 (in the case when the dust comes from iron-nickel bodies) and may have a minimum density on the order of 0.05 g/cm^3 ("dust balls"). The masses of the particles which move in circular orbits have a lower limit approximately equal to $4 \cdot 10^{-10}$ g at a density of 0.05 g/cm^3 and approximately $7 \cdot 10^{-10}$ g with a density of 3.5 g/cm^3. Their relative velocity is less than 20 km per second (Jaffe, Rittenhouse, 1962). The particles of dust beyond the limits of the Solar System, according to Shalen, have an average diameter of $5 \cdot 10^{-6}$ cm, rarely exceeding 10^{-5} cm. Their average density is $5 \cdot 10^{-26}$ g/cm^3. In terms of its chemical composition, cosmic dust may be considered to be similar to meteorites. All of the meteorites that strike the Earth essentially fall into the category of stony meteorites which contain 36% oxygen and 26% iron and smaller amounts of silicon, magnesium, and aluminum, and iron meteorites, consisting of 90% iron and about 9% nickel (Ovenden, 1959).

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TABLE 3. EXPERIMENT WITH SHEETS OF STAINLESS STEEL

Variant	Repe-tition	No. of Germinating Spores on Sheet	
		Nominal	% of Control
Control (without irradiation)	1	1040050	--
	2	1200570	--
Irradiation for 1.5 hrs, dose of $4.2 \cdot 10^8$ ergs/cm^2	1	71	0.0063
	2	73	0.0065
Irradiation for 3 hrs, dose of $8.4 \cdot 10^8$ ergs/cm^2	1	29	0.0025
	2	45	0.0041
Irradiation for 4.5 hrs, dose of $1.26 \cdot 10^9$ ergs/cm^2	1	63	0.0056
	2	23	0.0024

NOTE: The number of spores, equal to 1120310, is taken to be 100%.

In order to determine how much of the substance of the meteorites is able to protect microorganisms against lethal doses of ultraviolet radiation, we prepared artificial micrometeorites from the dust of the stony Kunashak meteorite (Imshenetskiy et al., 1966). As the binder, we used a 6% solution of soluble starch containing spores of *B. megaterium*. Such micrometeorites, "sharpened" with spores, have a conical shape. The height of the cone is from 36 to 145 microns; the base measures from 0.15·0.15 mm to 1·1 mm. In these experiments, beneath a protective layer of meteoritic substance measuring from 36 to 145 microns, spores of *B. megaterium* retained their viability under doses of ultraviolet radiation from $2.6 \cdot 10^7$ ergs/cm^2 to $7.8 \cdot 10^8$ ergs/cm^2. /133 These are enormous doses if we consider that the magnitude of the energy at a distance from the Sun equal to the distance from the Sun to the Earth in the range of wavelengths from 2,500-3,000 Å in a year, including solar flares, is equal to $5 \cdot 10^{11}$ ergs/cm^2.

Later, we made attempts to establish the minimum thickness of the metal shield which could protect microorganisms against the bactericidal doses of ultraviolet radiation (Imshenetskiy et al., 1967). As the protective material, we chose chromium. The technique of applying it to the spores of microorganisms was the same as for atomizing preparations for electron microscopy. A suspension of *Bacillus cereus* spores was applied to glass slides so that a layer on the glass consisted of a single row of cells (checked under the microscope). Then the layer of spores was covered with a film of chromium whose thickness varied. The spores, covered with a layer of chromium, were subjected to the action of ultraviolet radiation for three hours (a dose of $7.8 \cdot 10^7$ ergs/cm^2). A drop of liquid meat-peptone agar was placed on the irradiated areas and the slides were then placed in a thermostat for 48 hours at 28°. In this manner, the ability of the spores to germinate was checked. As controls, spores were grown which were covered with a film of chromium but were not irradiated with ultraviolet light.

As we can see from Table 4, in experiments where the thickness of the chromium film was less than 788 Å the growth of spores of *B. cereus* did not take place following irradiation. Chromium films 788 Å and more thick protected the spores of *B. cereus* against harmful effects of ultraviolet light. /134

TABLE 4. EXPERIMENTS WITH SPORES OF *BACILLUS CEREUS*, COATED WITH CHROMIUM FILMS

Thickness of Chromium Film, Å	Chromium Film		Without Film	
	Irradiation	Without Irradiation	Irradiation	Without Irradiation
200	-	+	-	+
408	-	+	-	+
445	-	+	-	+
446	-	+	-	+
541	-	+	-	+
594	-	+	-	+
595	-	+	-	+
670	-	+	-	+
788	+	+	-	+
818	+	+	-	+
820	+	+	-	+
1040	+	+	-	+
1160	+	+	-	+
1507	+	+	-	+
1566	+	+	-	+
1606	+	+	-	+
1712	+	+	-	+
2600	+	+	-	+

NOTE: +, growth of *B. cereus*; -, absence of growth of *B. cereus*.

Both according to the calculations of Arrhenius and according to those of Sagan, given below, particles of very small size could wander in interplanetary space under the pressure of light rays. This may be why, when the action of space factors on viable embryos is evaluated, no attention is given to the problem of possible protection of them agains ultraviolet radiation. The results of our experiments with chromium films has shown that particles like those about which we are speaking can provide such protection. As a matter of fact, a sphere with a diameter of approximately 0.6 micron made of a material similar to an iron meteorite with a wall thickness on the order of 800 Å could be a reliable protector for a microbe whose diameter did not exceed 0.4 micron. At the present time, such microorganisms are known. Some of the smallest living cells are the representatives of the order Mycoplasmatales. These bacteria measure from 0.1-0.15 micron to 0.2-0.7 micron (Nikitin et al., 1966). These also include the pleuropneumonia-like organisms (PPLO) and

the filtrable forms of ordinary bacteria. According to the data of Morovitts and Turtelott (1964), more than 30 strains of PPLO have been isolated from soil, drainage water, etc. Several representatives of the genus *Micrococcus* (Krasil'nikov, 1949) are also smaller than 0.2-0.5 micron (*Micrococcus subtilis* -- 0.3-0.5 micron; *M. minimus* -- 0.2-0.3 micron; *M. aquatilis* -- 0.3 micron). Usually, dimensions from 0.15-0.5 -- 1.0 micron to 2 -- 3-15 -- 20 microns are given for known bacterial organisms (Nikitin et al., 1966). However, this is valid from microbes which grow on nutrient media under laboratory conditions. In nature, salt microorganisms have much smaller sizes.

Hence, it follows from all of the above that it is comparatively easy to protect living cells against the effects of ultraviolet light, so that we cannot consider this extremal factor of the space environment to be critical in an evaluation of the possibility of transporting viable microorganisms from one planet to another. In this connection, we also cannot include the space medium as a sterilizing agent, and there is therefore no basis for eliminating sterilization of the surfaces of spacecraft.

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METEORITES AND LIFE

G. P. Vdovykin

ABSTRACT. Study of the nature of hydrocarbons, amino acids, and large-molecule organic compounds in chondrite meteorites from the viewpoint of the origin of life on Earth. It is shown that organic compounds in meteorites are formed by abiogenetic reactions. Also, no fossilized microorganisms were detected in meteorites. Possible ways of seeding life on Earth by meteorites, and the possible contribution of meteoritic organic compounds to the formation of life on Earth are discussed.

The problem of the origin of life has acquired considerable timeliness recently in conjunction with the extensive experimental studies of the heavenly bodies. Meteorites are particularly important in this regard.

Recent detailed studies of meteorites have shown that they contain complex organic substances of a radiogenic nature which have certain similarities to terrestrial organic compounds. The same substances can be obtained synthetically from simple original compounds when they are irradiated or heated. In meteorites, carbon has other forms in which it occurs, the most interesting of which is diamond. /136

On the basis of the results of a study of meteoritic carbonaceous substances, a new branch of science has grown up -- the cosmochemistry of carbon, dealing with two basic problems -- the origin of organic compounds in the Solar System and the origin of extraterrestrial diamonds. The first of these is simultaneously the basis for the problem of another new science -- exobiology, which studies the possible forms of existence of extraterrestrial life. The other problem of cosmochemistry of carbon is simultaneously linked to the problem of collisions between large heavenly bodies.

In this paper, we shall discuss some of the problems of exobiology and the origin of life on Earth, in which we may be aided by the results of a study made by Vdovykin (1967) dealing with meteoritic organic compounds.

Nature of Organic Compounds of Meteorites

Carbonaceous chondrites are enriched with organic compounds; they are rare stony meteorites, of which about 30 have been found so far.

The first carbonaceous chondrite (Ale) fell in France in 1806; it weighed 260 grams. The most recent carbonaceous chondrite, Revelstouk, weighing one gram, fell in Canada in 1965. In our country, carbonaceous chondrites have fallen also: Groznaya (in 1861), Migei (in 1889), Staroye Boriskino (in 1930). When they fly through the atmosphere in the braking region at an altitude of about 10-20 km, the meteorites break up into several pieces. Hence, a meteorite frequently consists of several individual examples (Figure 1).

Carbonaceous chondrites differ from other meteorites in the high content of carbon (up to 4.6%), the presence of water, included in the lattice of the iron-magnesium silicates (the chlorite-serpentine type), the presence of carbonates, native sulphur and other characteristics of their chemical, mineral and structural composition. On the basis of these characteristics, Wiik (1956) divided carbonaceous chondrites into three types (groups): Orgey, Cold Bokkeveld, Felix.

The author studied the organic substances in the Orgey, (type I), Migei, Staroye Boriskino, Cold Bokkeveld, Marrey (type II) and Groznaya. The chemical composition, structural features, nature and origin of meteoritic organic compounds are discussed in detail in a monograph (Vdovykin, 1967).

The content of organic compounds in carbonaceous chondrites reaches 7%, with about one-tenth of them soluble in organic solvents. This is the so- /137
called bituminous substance. The remainder of the organic matter cannot be extracted by solvents, and have therefore been only slightly investigated not only in meteorites but in ores.

The complex mixture of organic substances is distributed more or less uniformly in terms of volume in carbonaceous chondrites which are between the grains of silicates or Donders. Frequently the carbonaceous material is also found inside individual silicate grains. In some instances, organic substances are found in meteorites of other types -- ordinary chondrites,

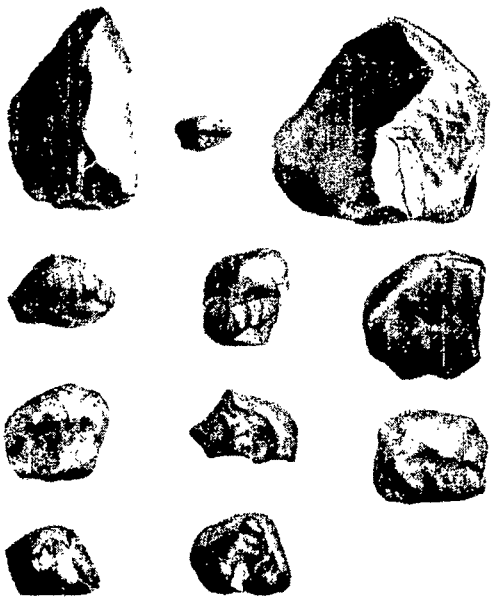

Figure 1. Orgey Carbonaceous Chondrite. Various individual examples from a meteorite shower in various positions. Considerably reduced.

diamond-containing achondrites ureilites, in the graphite nodules of iron meteorites.

The composition of organic substance making up carbonaceous chondrites has been found to contain paraffin aromatic hydrocarbons, and oxygen-containing compounds (Vdovykin, 1967). It has been shown (Meinschein, et al., 1963) that the hydrocarbons are a complicated mixture of compounds. Recently Studier et al., (1968) and Oro et al., (1966) studied the hydrocarbons in carbonaceous chondrites with the aid of a combination gas-chromatograph and mass spectrometer, the LKB-9000, whose resolving power is 1,000, whose range of masses is 1,200, with a sensitivity of 0.01 microgram for a solution and 0.1 microgram for a solid. They showed that the distribution of hydrocarbons in carbonaceous chondrites is somewhat similar to the distribution of hydrocarbons in ancient sedimentary rocks, and also in the products of abiogenic synthesis, which Studier et al., (1968) produced from CO and H_2 according to Fisher-Tropsh (Figures 2 and 3). /138

In all of the carbonaceous chondrites which were studied by Oro, isoprenoid hydrocarbons were found. The most frequent of them were pristane, phytane, norpristane ($0.35\text{-}8.10\cdot10^{-4}\%$). The total content of alkanes increased in the direction of the carbonaceous chondrites of the III type. It is felt that the isoprenoids are products of conversion of steroids which enter into the composition of living substance. Therefore, the question has arisen as whether or not isoprenoids in carbonaceous chondrites are an indication of contamination of these meteorites under terrestrial conditions. As a matter of

fact, in the Groznaya meteorite, for example, an unusually high content of alkanes was found, including isoprenoids, $412 \cdot 10^{-4}$% (Oro et al., 1966). The author of the present article sent Oro a little piece of the Groznaya meteorite, taken from the internal part of the large sample of the meteorite for additional testing of the purity of the sample of meteorite which the author examined (Vdovykin, 1967). This sample of the Groznaya meteorite was also found to contain isoprenoids (Oro, Gelpi, 1968), but their quantity ($7 \cdot 10^{-4}$% alkanes) fell within the limites of variation of the content of isoprenoids in other meteorites of this group, i.e., the sample of the Groznaya meteorite which was sent was not contaminated under terrestrial conditions. The isoprenoid hydrocarbons, as was demonstrated experimentally by Studier et al. (1968) may have been formed by means of inorganic synthesis (Figure 4). /139

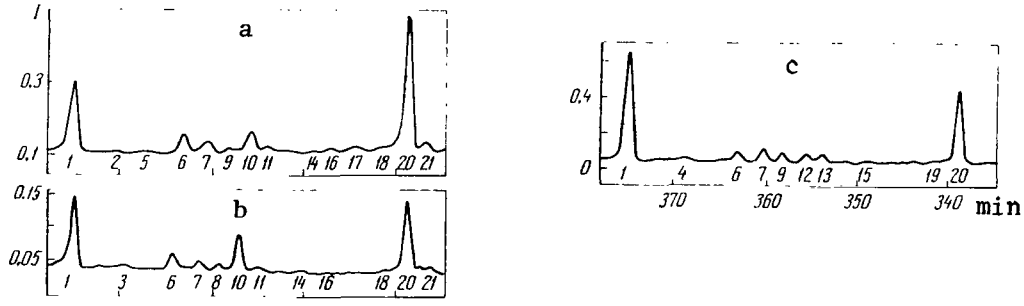

Figure 2. Spectrograms of The Hydrocarbon Series C_{14}-C_{15} In a Benzene Extract From the Orgey Meteorite (a); In Products Isolated During Heating to 200°, Marrey Meteorite (b); In a Synthetic Product Formed by the Fisher-Tropsh Reaction, $CO+5_2^D$; 150-350° (c) (Studier et al., 1968).
I, Intensity of peaks at the output of an electronic multiplier; 1, n-C_{15}; 2, Olefins C_{15}; 3, Olefins (C_{15}?); 4, Dimethylnaphthaline; 5, Olefins; 6, Trimethyltetradecane (C_{15}); 7, 2-methyltetradecane (C_{15}); 8, Branched paraffins (C_{15}); 9, 2,3-dimethyltetradecane (C_{15}); 10, 2,5-dimethyltetradecane (C_{15}?); 11, Olefins, C_{15}, 12, 3,4-dimethyltetradecane (C_{15}); 13, 4,5-dimethyltetradecane (C_{15}); 14, Olefins C_{15}; 15, Olefins; 16-18 Olefins C_{15}; 19, Branched paraffins, C_{15}; 20, n-C_{14}; 21, Olefins, C_{15}.

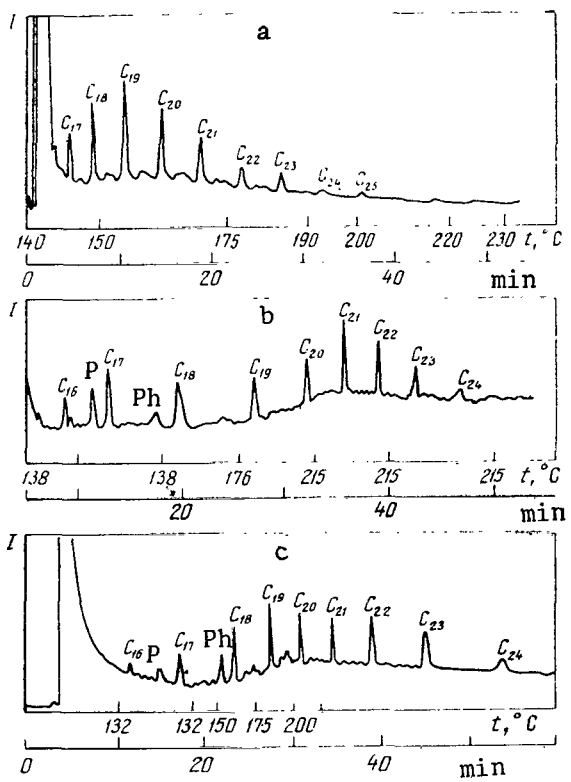

Figure 3. Chromatograms of Alkanes Isolated From The Orgey (a) and Migei (b) Meteorites (Oro et al., 1966) and Alkanes Isolated From Shales of the Gunflint Formation (1.9 billion years) (c) (Oro et al., 1965).
P = Pristane; Ph = Phytane.

In addition to the hydrocarbons in the carbonaceous chondrites, various investigators have found aromatic and fatty acids, as well as organic substances which contain sulphur and chlorine (thiophenes, alkylchlorides, etc.), amino acids and carbohydrates, nitrous cyclic compounds (purins, triazines).

The author studied amino acids in the carbonaceous chondrites called Migei, Staroye Boriskino and Groznaya. Samples of the meteorites weighing 2 grams, taken from the internal portion of large samples, were treated with 2 N HCl and HF after being pulverized, and then heated slightly for a long time to free the carbonaceous material. The residue was hydrolyzed for 24 hours with 6 N HCl at 110°. The author studied the hydrolysates for their content of amino acids using the paper chromatography method (mobile solvent: n-butyl alcohol -- acetic acid -- water, 4:1:5). Following staining with ninhydrin, a series of amino acids appeared on the chromatograms. The Migei meteorite was found to contain 9 amino acids: lysine, histidine (basic), aspartic and glutaminic acid (acid), glycine, alanine, serine, valine, treonine and also traces of leucine (neutral). The Staroye Boriskino meteorite was found to contain lysine, aspartic and

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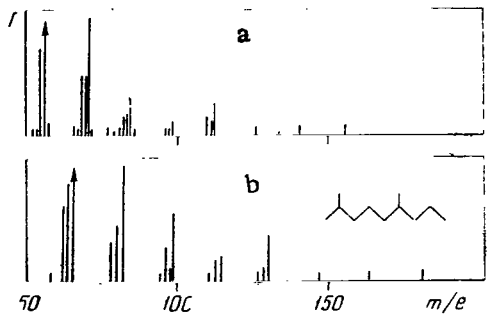

Figure 4. Mass Spectra of Isoprenoid Hydrocarbon, $C_{11}H_{24}$ of the Marrei Meteorite (a) and Deuterated Isoprenoid Hydrocarbon $C_{11}D_{24}$, Synthesized from CO and D_2 (catalyst -- iron meteorite, Canon Diablo (b).

I, Relative Intensity of Peaks.

glutaminic acids, glycine, alanine, serine, valine and treonine. The Groznaya meteorite contained six amino acids: aspartic and glutaminic acis, glycine, alanine, serine, and valine. In all three meteorites, such amino acids as glycine (glycocoll) and alamine (Figure 5) were most plentiful. This agrees with other data on the distribution of amino acids in meteorites (Kaplan et al., 1963).

For a comparison, the author studied amino acids in several terrestrial natural formations. Petroleum from the Akhtyrsko-Bugundyrskiy deposit in the northwestern foothills of the Caucasus (No. 3 well, Sarmatian) was found to contain the following amino acids (in order of decreasing content): aminobutyric acid, valine, alanine, glutaminic acid, aspartic acid, lycine, histadine and glycine. Approximately the same order of reduction in content of amino acids was observed in other petroleums (Degens, Bajor, 1960), with 10 amino acids being found.

The stratal water in the northwestern foothills of the Caucasus (Labinsk, Well No. 1, Cretaceous, depth of 2258 m) contains the following amino acids: histadine, aspartic acid, valine, glutaminic acid and probably amides as well. Only three amino acids (alanine, glutaminic acid and histadine) have been found in the Karelian Schungite shale. /141

Hence, a great many different organic substances have been identified in carbonaceous chondrites, including a number of individual compounds. It was decided to check for the presence of porphyrins in the meteorites. A study of the benzene eluate of the Orgey meteorite revealed that it may contain vanadium-porphyrin complexes with a relative content of 10^{-6}% (Hodgson, Baker,

1964). In studying the Migei meteorite, we were unable to find in it the existence of porphyrin pigments (Vdovykin, 1967).

We were particularly interested in the high-molecular organic compounds in the carbonaceous chondrites -- polymer substances that were insoluble in organic solvents. The author isolated them by treating meteorite powder with HF and HCl with weak prolonged heating (Vdovykin, 1967). The content of these substances decreased from carbonaceous chondrites (Type I) to meteorites of type III. The structural studies which we performed indicated that the degree of carbonization of the compounds represented by the high molecular substances with condensed aromatic structure increased in the same direction. Their infrared spectrum (Figure 6) showed absorption bands at 1080-1175 cm^{-1}, caused by aromatic C-H bonds at 1440 cm^{-1} (the deformed OH group of the carboxyl or alcohols) and 1660 cm^{-1} (variations -- C = O, probably in the carboxyl group or variations connected with the aromatic group). The infrared absorption spectrum of this substance, isolated from the Migei meteorite, is quite similar to the absorption spectrum of kerogen, removed from ancient shales. In contrast to terrestrial organic compounds, the high-molecular organic substance of carbonaceous chondrites, like bituminous substance, contains chlorine. For example, the organic substance of the Staroye Borinskino meteorite, according to our data, contains 2.56% chlorine (Vdovykin, 1967). Recently Raya (cited in Hayes, 1967) isolated polymer organic substance in a similar fashion from the Orgey meteorites and determined its elementary composition (in %): C -- 70.39, H -- 4.43, Cl -- 1.22, F -- 1.25, N -- 1.59, S -- 6.91, O -- 9.80 (direct determination), ash -- 4.58.

Organic compounds in meteorites, as we see, have certain similarities to terrestrial organic substances of biogenic nature and to products of abiogenic synthesis.

As products of abiogenic synthesis, organic compounds in meteorites are optically inactive.

1. The organic compounds entered the meteorites under terrestrial conditions, i.e., these substances have a terrestrial biogenic nature. The enrichment of the carbonaceous chondrites alone with organic compounds and

several differences in the composition of organic substances in meteorites from terrestrial biogenic compounds do not contradict the contamination origin of the meteoritic organic substances.

2. The organic substances in meteorites were formed as a result of extraterrestrial biogenic processes. Modern data on the structure of meteorites indicate that the bodies from which the meteorites were originally formed were of small size. The asteroids lack conditions favorable for the development and evolution of living matter. The composition of the organic substance in meteorites, in contrast to biogenic compounds, is simpler and optically active substances have not been reliably determined in them. A number of other peculiarities of meteoritic organic compounds also fail to support their extraterrestrial biogenic nature. /143

3. Organic compounds in meteorites were formed as the result of chemical processes during the formation of all meteoritic substance. Extraterrestrial radiogenic nature of meteoritic organic compounds is supported by the similarity of these substances to the products of laboratory synthesis, and increased content of deuterium, interaction of organic substance and mineral phases in carbonaceous chondrites.

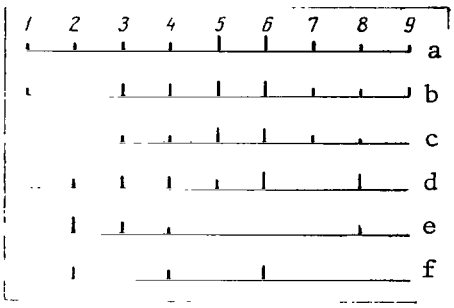

Figure 5. Relative Content of Amino Acids in Hydrolysates from Carbonaceous Meteorites Migei (a), Staroye Boriskino (b), Groznaya (c) and Some Natural Terrestrial Objects: Petroleum (d), Stratal Water (e) and Schungite (f): 1, Lysine; 2, Histidine; 3, Aspartic acid; 4, Glutaminic acid; 5, Glycine; 6, Alanine; 7, Serine; 8, Valine; 9, Treonine.

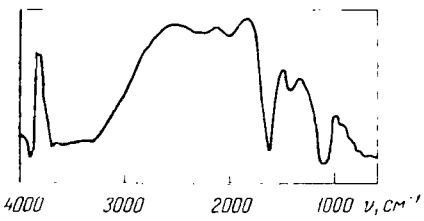

Figure 6. Infrared Absorption Spectrum of High-Molecular Organic Substance Isolated From the Migei Meteorite.

Do Meteorites Provide Evidence of Extraterrestrial Life?

Under terrestrial conditions, as we know, the process of conversion of sediments into rock involves the fossilization of the organic remains contained in them. The methods of biostratigraphy are based on the results of study of the fossilized petrified remains. Are there similar fossilized remains in meteorites?

In studying the organic matter in carbonaceous chondrites, the author found that the dark carbonaceous material, opaque under the microscope, contained luminous (in ultraviolet light) round microinclusions of bituminous material (Vdovykin, 1960, 1965, 1967).

Claus and Nagy (1961), on the basis of the morphological similarity, described microinclusions which were luminous in ultraviolet light from the Orgey and Ivuna meteorites as remains of fossilized microorganisms from outer space. These "organized elements", as described by the investigators, had a round, cylindrical, hexagonal shape and measured from 4 to 30 microns. There were 1700 particles per milligram of the meteorite sample. On the basis of the morphological features and the reactions to staining, Claus and Nagy suggested that the meteorites contained extraterrestrial life forms. Later, Nagy and other investigators performed a number of additional analyses. The nature of the "organized elements" evoked considerable discussion in the literature.

The author performed a morphological study of the "organized elements" using the Migei meteorite (Vdovykin, 1964). Powder from this meteorite was centrifuged in liquid with different densities (chloroform, bromoform-acetone, bromoform). The fractions were found to contain grains of silicates which had a rounded shape and measured up to 64 microns. Frequently the grains were wrapped in carbonaceous substance imitating formed elements. The particles which luminesced in ultraviolet light were concentrated in the fractions with a density of 1.5-2.88. This consisted primarily of the bituminous inclusions. The fraction with this density in the tests performed by other authors may have been subjected to terrestrial contamination. Consequently, /144
the microparticles described as "organized elements" are mineral grains that

belong to the meteorites but are also the result of random contamination under terrestrial conditions. We have no basis for considering that meteorites carry traces of extraterrestrial life.

In addition to the direct indications of extraterrestrial life -- fossilized microorganisms -- there may be indirect indications, in particular, the presence of free organic radicals.

Free organic radicals -- are particles of molecules that have unpaired electrons in their structure, which cause paramagnetic characteristics of the particles. Free radicals are characteristic of living matter. It has been established that various processes of conversion of organic compounds in nature take place with their participation. They have been found in petroleum, coal, peat, etc. In these substances, the stability of the stable free radicals is determined by the high degree of dislocation of the unpaired π-electrons inside the complex aromatic structure. However, stable free radicals are also contained in carbonaceous substances whose formation does not involve biogenic activity.

In our study involving the use of the method of electron paramagnetic resonance (EPR), free radicals were found in the composition of high-molecular organic substance from the carbonaceous chondrites of the Orgey, Migei, Staroye Boriskino, Cold Bokkeveld and a number of other carbonaceous materials.

In the EPR spectra, rather intensive lines of paramagnetic absorption with similar parameters are found in meteorites; their g-factor is 2.002-2.003, $\Delta H \approx 6$ units. This indicates that unpaired π-electrons are dislocated in the complex aromatic structure of high-molecular organic substance in meteorites.

Consequently, the free radicals develop simultaneously with the formation of the entire polymer organic substance in carbonaceous chondrites, i.e., at an early stage of development of the material under the influence of the same energy source in the process of conversion (irradiation, heating).

In a further stage of existence of carbonaceous chondrites, following the breakup of the parent bodies, their material was first irradiated with hard cosmic and solar rays. The organic substance in the meteorites may then have been altered partly and the concentration of free radicals increased.

To test this theory, we performed an experiment involving irradiation with protons having an energy of 600 MeV, applied to 5 grams of brea, containing 8.11% organic substance, from the asphalt deposit at Asphalt Mountain (northwestern foothills of the Caucasus, Maykop). The sample was irradiated in the synchrocyclotron at the Combined Institute of Nuclear Study (Dubna), using an extracted proton beam. The absorbed dose of radiation was $1 \cdot 10^8$ rad. /145

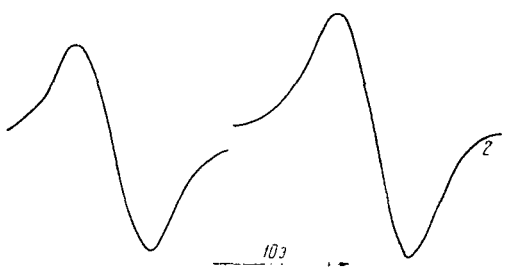

Figure 7. Increase in the Concentration of Free Radicals in Organic Substance From Brea (from the Asphalt deposit in the northwestern foothills of the Caucasus), Irradiated with Protons (Dose = $1 \cdot 10^8$ rad).
1, Spectrum of electronic paramagnetic resonance of organic substance before irradiation with protons; 2, Ditto, after irradiation.

Prior to irradiation, the component composition of the organic matter extracted with chloroform was as follows: oils -- 60.8%, resins -- 17.7%, asphaltenes, 21.5%. After irradition of the sample with protons, the component composition was slightly different: oils -- 65.9%, resins -- 16.9%, asphaltenes -- 17.2%. Consequently, the radiation was followed by a slight increase in the oil content at the expense of the resin and asphaltene components. The concentration of free radicals in the organic substance of the investigated sample increased somewhat following irradiation, as indicated by the more intensive signal received on the EPR spectrum in comparison to that received prior to the radiation of the sample (Figure 7).

This experiment supports the possibility of an increase in the concentration of free radicals with high-energy irradiation of organic substances.

Hence, the stable free organic radicals in the carbonaceous chondrites are not related to the biogenic activity of the parent body of the meteorite.

As a matter of fact, the free radicals of different composition are present in many space objects. As a rule, they are found in the composition of the Sun and other stars, interplanetary substance and in the makeup of comets.

The Role of Meteorites and Comets in the Origin of Life on Earth

The presence of organic compounds of radiogenic origin in meteorites forces us to consider the question of the arrival of complex organic substances on Earth from space and the development on the basis of these compounds /146
of living matter. It has been suggested (Briggs, 1963), that the organic compounds which reached the Earth in the form of carbonaceous chondrites may have great significance in the general content of hydrocarbons in the Earth's crust.

The 30 carbonaceous chondrites which are known at the present time weigh about 132 kg. If we estimate the arrival of carbonaceous chondrites all during the time of existence of the Earth ($5 \cdot 10^9$ years) as being regular, then with an average content of organic compounds in the carbonaceous chondrites approximately equal to 4%, 10^{11} organic compounds must have arrived on Earth during the time of its existence in the form of carbonaceous chondrites. This number is not underestimated here due to the probability of unrecorded falls of carbonaceous chondrites during the last 150 years. The amount of hydrocarbons in the Earth's crust, for example, according to the calculations of Kalinko (1964) amounts to about $15 \cdot 10^{19}$ grams. Consequently, the contribution from meteoritic organic substances is small.

Another possible source for the arrival of organic substances from space on Earth is comets. According to the current model, comets consist of a conglomerate of solid mineral particles, ice from frozen gases (H_2O and NH_3, CH_4, CO_2, C_2N_2) and frozen free radicals. Spectroscopic studies of the heads of comets have revealed CN, C_2, C_3, CH, NH, NH_2, OH (Figure 8). The spectra of comet tails have been found to contain the following gases: CO^+, N^+_2, OH^+, CO^+_2, CH^+.

Comets are considered to be the earliest and least changed bodies in the Solar System. It is probable that in the entire history of mankind there has been only one encounter of the Earth with a comet -- the Tungus meteorite in

1908. The possibility of similar phenomena at earlier times cannot be excluded, especially during the early stages of development of the Earth. Oro (1961a) made a very interesting calculation which showed that during the first $2 \cdot 10^9$ years of existence of the Earth collisions with comets added 10^8-10^{12} tons of cometary material. If we assume that 10-30% of this substance consisted of carbonaceous compounds, the total amount would be 10^{13}-10^{17} grams. However, this value is quite insignificant in terms of the total quantity of carbonaceous material on Earth.

Unquestionably, meteorites and comets have contributed carbonaceous material to Earth. These compounds, together with terrestrial carbonaceous substances, have entered the cycle during the process of development of the Earth and have passed through living substances. We cannot distinguish these added substances from terrestrial carbonaceous compounds. The extensive distribution of carbon and other volatile elements (H, O, N, S, etc.) in space leads us to think that these elements were present in the terrestrial material at the early stages of its development in the composition of various compounds. In addition, in the process of zone melting of terrestrial materials and degassing of volatile compounds, they have been transported to /147
the upper part of the Earth's crust, where they have been exposed to the influence of radiation and radiogenic synthesis of complex organic substances may have taken place (Vinogradov, 1967).

Many experiments (on whose results a new branch of science has been built, called experimental organic cosmochemistry) indicate that complex organic substances may be formed from simple original compounds with participation of various energy sources. The conversion takes place in stages, with participation of intermediate reaction products and especially free radicals. For example, the formation of the purine base of adenine when HCN is heated takes place, as Oro (1961b) states, according to the following scheme (Figure 9).

Could not the passage of meteorites through the Earth's atmosphere be significant as far as the origin of life is concerned, as an energy source through which simple components of the early terrestrial atmosphere were

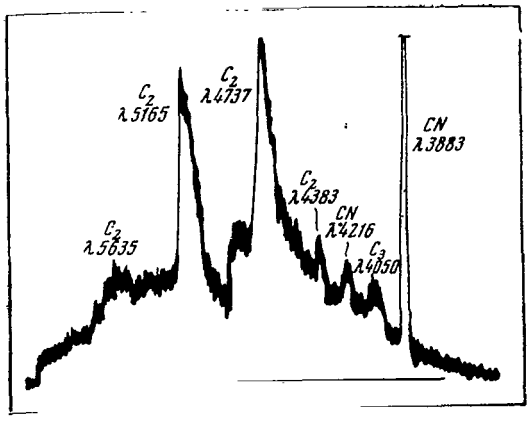

Figure 8. Photoelectric Recording of the Spectrum from the Head of The Arend-Roland Comet of 1956h (Brandt, Hodge, 1964). Band width 42 Å, scanning rate 270 Å/min, recording made on 29 April 1957.

synthesized into complex organic substances? Hochstim, (1963) suggested that the complex organic compounds in the terrestrial conditions may have been formed as a result of the action of a shock wave during the fall of enormous meteorites at supersonic speeds as they fell through the atmosphere or during the interaction of the meteoritic substance with water if the meteorite falls into water. Hochstim supported this /148 theory experimentally. He fired a shot through a mixture of water and gas (composition of the gaseous medium 40% CH_4, 40% NH_3, 20% H_2); the speed of the bullet was 4.6 km/sec. In the water after the shot, organic substances were found whose molecular weight reached 200. But this energy contribution was not very great. The principal sources of energy in the process of conversion of primary terrestrial carbonaceous substances must have been solar (ultraviolet) and cosmic radiation, heating during various exothermal reactions, radiation from the decay of radioactive isotopes, etc.

Consequently, the carbonaceous substance of meteorites and comets did not play an important role in the origin of life on Earth.

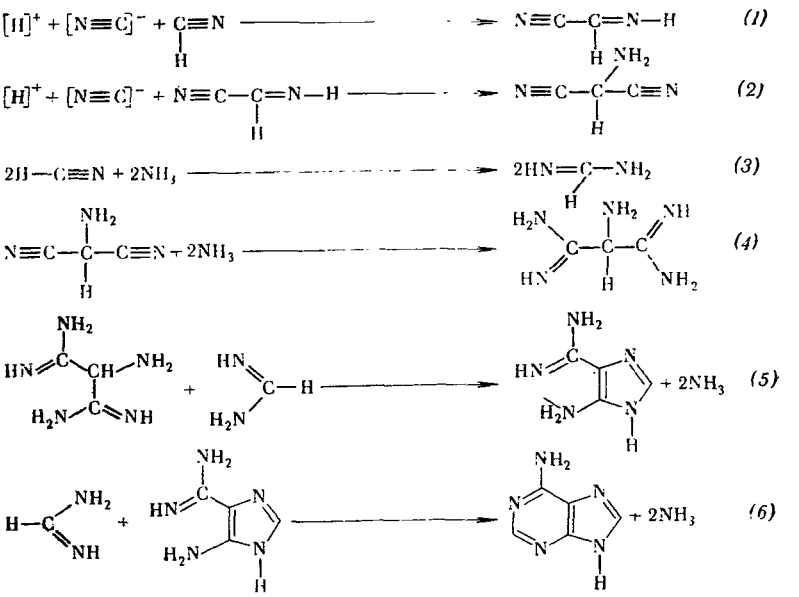

Figure 9. Mechanism of the Synthesis of Adenine from HCN (5 HCN → Adenine)

Meteorites as Indicators of the Medium for the Development of Living Matter on Earth

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A medium was required for the development and subsequent evolution of living matter under terrestrial conditions; it consists of the atmosphere and the hydrosphere (Oparin, 1968). The origin of these envelopes is explained, in the view of Vinogradov et al. (1967), by the degassing of the Earth's mantle, whose substance is similar to the silicate phase of stony meteorites and chondrites. The carbonaceous chondrites may be of considerable importance in a discussion of these problems.

Structural details of these meteorites indicate that their substance was altered somewhat by secondary processes, with these changes taking place under the influence of volatile compounds. Many investigators feel that other types of stony meteorites and the planets of the Solar System were formed from a substance similar to carbonaceous chondrites.

Carbonaceous chondrites are rarely enriched with volatile elements, which enter into the composition of minerals which are characteristic of only this

group of meteorites. The content of characteristic "low-temperature" minerals changes in different meteorites, increasing at the same time as the content of carbon increases and the amount of organic substances. Meteorites of the Orgey type consist almost completely of minerals that are characteristic of carbonaceous chondrites.

The composition of these minerals in the downed state includes carbon (carbonates), hydrogen (in the form of constitutional water in minerals of the chlorite-serpentine type), sulphur (in the form of elementary sulphur and sulphates), nitrogen (ammonium salts), oxygen (in the form of chlorite-serpentine minerals and nickel-containing magnetite), with these minerals being in combination with the ordinary "high-temperature" minerals found in other chondrites (olivine, pyroxenes, troilite FeS, nickeliferous iron -- kamasite, chromite, etc.). Carbonaceous chondrites exhibit a lack of equilibrium both in the mineral and in the chemical composition, i.e., the chemical composition of individual minerals in them differs somewhat.

In studying carbonaceous chondrites in petrographic sections, it is apparent that the minerals which are characteristic of these meteorites have secondary involvement of "high temperature" minerals. This is especially evident in the distribution of minerals of the chlorite-serpentine type, which were found in carbonaceous chondrites by Kvasha (1948). The chondrules in carbonaceous chondrites are frequently "pitted" on the periphery and replaced by aqueous silicates. Therefore, the size of the chondrules in these meteorites is smaller than in ordinary chondrites, and in some carbonaceous chondrites the chondrules are even lacking, having been completely destroyed. In other carbonaceous chondrites, the grain of the olivine is sometimes preserved at the center of the chloritic chondrule (Vdovykin, 1967). Frequently, /150 however, the chondrules are completely replaced, and inclusions of carbonaceous substance can be seen in the chondrules (Figure 10). Consequently, the processes of replacement of the chondrules and the penetration of carbonaceous substance were simultaneous. Least frequently, "low-temperature" minerals are distributed in carbonaceous chondrites containing minimum amounts of carbon.

Figure 10. Chondrule Consisting of Aqueous Iron-Magnesium Silicates in the Staroye Boriskino Meteorite (Vdovykin, 1967).

The chondrule is located on a black carbonaceous substance which has penetrated the chondrules. Section No. 191, 450 X.

The distribution of volatile elements that enter into the composition of "low-temperature" minerals indicates that the replacement of the substance of the carbonaceous chondrites took place with low-carbon carbonaceous chondrites changing to high-carbon types. This replacement must have taken place at the early stages of development of the meteoritic substance.

Prior to the agglomeration of the substance of the gas-dust cloud, the volatile components were in a frozen state, probably in the form of stable compounds of the type H_2O, CO_2, CH_4, NH_3, stable reactive compounds (H_2O_2, C_2H_2, C_2H_4 etc.) and frozen free radicals (OH, CN, etc.). During the process of agglomeration on the bodies, these substances must have penetrated the upper zones of the asteroidal bodies at a somewhat increased temperature, acting intensively on the mineral substance. Since the effusion of the volatile components took place in the interchondrular space, they must have acted first of all on the interchondral substance. The composition of these gases included oxygen-containing compounds: their oxygen oxidized the principal mass of the carbonaceous chondrites. In the surface zones of the asteroids, where there may have been an ice shield, the concentration of volatile components increased and the process of oxidation became more intensive. This is indicated by the oxidized state of the interchondrular substance in meteorites of the III type and the maximum degree of oxidation in carbonaceous chondrites of the I type. The process of replacement of the substance must have taken place with the participation of

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many gases, since the effect of the gases on the mineral components was not uniform.

In the course of such a complex interaction of mineral phases in volatile components, there was probably an isotopic exchange as indicated by the results of the determination of the isotopic composition of volatile elements in carbonaceous chondrites.

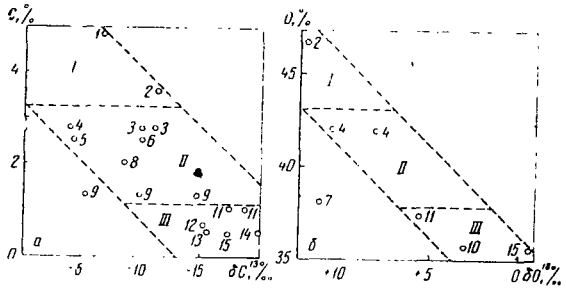

Figure 11. Isotopic Composition of Carbon (a) and Oxygen (b) as a Function of the Total Content of Carbon and Oxygen in Carbonaceous Chondrites Types I, II and III.
Meteorites: 1, Ivuna; 2, Orgey; 3, Migei; 4, Marrey; 5, Santa Cruz; 6, Navapali; 7, Al Rais; 8, Staroye Boriskino; 9, Cold Bokkeveld; 10, Vigarano; 11, Mokoya; 12, Kinsaz (carbonaceous meteorite); 13, Lance; 14, Groznay; 15, Felix.

The isotopic composition of volatile elements, particularly carbon and oxygen, differs in different carbonaceous chondrites. As in the discussion of all other characteristics of carbonaceous chondrites, here we can see a clear relationship between the three types of carbonaceous chondrites. The results of a determination of isotopic composition of the carbon in the carbonaceous chondrites, performed by Boato (1954) and our results of the investigation of the isotopic composition of carbon in a number of carbonaceous chondrites (Vinogradov et al., 1967) indicate that as the quantitative content of carbon increases in isotopic composition, it grows heavier (Figure 11).

Recently Begemann and Heinzinger (1968) studied the isotopic composition of the total carbon in dark and bright sections of ordinary meteorites (Pantar and Breitscheid). These authors showed that the dark areas of the meteorites, enriched with carbon, contained more "weighted" carbon than the

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bright parts. In the Pantar meteorite, the dark areas contain up to 2% carbon, while the δC^{13} varies from -15.6 to -20.8%; in the bright areas, the carbon amounts to 0.07-1.2%, while the value of δC^{13} varies from -19.2 to 21.8%. In the dark areas of the Breitscheid meteorite, the carbon content is 0.47-0.78%, δC^{13} is 20.5%; in the bright parts, the carbon content is 0.21-0.40% while δC^{13} is -24.3%. The enrichment of the oxygen with the heavy isotope O^{18} in carbonaceous chondrites (Taylor et al., 1965) also takes place simultaneously with an increase in the content of carbon and oxygen (Figure 11). Consequently, there was a predominant loss of volatile isotopes of carbon and oxygen during the increasing secondary replacement of the substance of carbonaceous chondrites and the enrichment with volatile elements during the development of these meteorites.

Some investigators suggest that carbonaceous chondrites may have been formed in the early stage of development of the Solar System, even before the agglomeration of asteroidal bodies, or else they may be of cometary origin (Studier et al., 1968). This makes it very difficult to explain the process of secondary replacement of their substance and the gradual change in the characteristics of their structure during the transition from the carbonaceous chondrites of the III type to the meteorites of the I type. In any case, the carbonaceous chondrites which are enriched with volatile elements are most suitable for evaluation involving the primary substance from which the heavenly bodies in our Solar System are formed.

In the process of agglomeration and subsequent differentiation of the material composing the Earth, the volatile elements in its composition must also have been transported to its outer zones. Later these elements, which partly remain in compound substances, must have been liberated in the course of local heating. They were a part of the composition of the upper layers of the Earth. Undoubtedly these elements entered into the composition of the outer layers of the Earth in the form of very simple compounds and were compressed by the gravitation of the planet due to its large size. The degassing of the mantle led to the formation of an atmosphere and hydrosphere. The favorable development of the planet as a whole -- the differentiation of

its substance with formation of the Earth's crust, degassing of the mantle with formation of the hydrosphere and atmosphere -- created conditions in the medium for the development and evolution of life.

Significance of Meteorites for Explaining the Origin of Life on Earth /153

The original terrestrial organic compound that existed in the early stages of development of the Earth was subsequently drawn into the cycle by the biosphere and completely converted by it. The carbonaceous substances of juvenile origin are found in the igneous rocks, and are ejected in large amounts by volcanoes. But these substances underwent their changes at the same time as the evolution of terrestrial material.

The presence of complex organic compounds of radiogenic origin in meteorites is of great significance for explaining several problems of the origin of living matter under terrestrial conditions. How were complex organic substances formed in meteorites?

The quantitative content of organic substances in carbonaceous chondrites increases gradually when moving from type III to type I meteorites. The degree of carbonization of high-molecular carbonaceous material decreases in the same fashion. The nature of the interaction of the organic compounds and the "low-temperature" mineral components indicates that the formation of both took place simultaneously in all probability.

It is obvious that during the agglomeration of asteroidal bodies the simple carbon-containing compounds that were present in the gas-dust cloud must have undergone some heating and entered into chemical reactions which took place simultaneously with the effusion of these compounds into the outer zones of the asteroidal bodies. The process of complication of organic substances must have been accompanied by the formation of more high-molecular substances. Therefore, a slight amount of carbonaceous material (especially polymer substances and paraffin hydrocarbons, whose bonding energy is higher than in the aromatic hydrocarbons) remained in a strictly fixed state in the intermediate zones of the asteroids, adsorbed on the surfaces of mineral grains.

The organic components of the upper zones of the asteroids are the result of two processes -- conversion of compounds that existed here earlier and were subjected to cosmic and solar irradiation, and conversion of volatile compounds, the products of distillation from deeper zones of the asteroids. These conversions took place with participation of free organic radicals, which were retained in the stabilized state in the carbonaceous chondrites until the present time.

The possibility is not excluded that the synthesis of complex organic compounds from simple substances took place even before the agglomeration of asteroidal bodies. During agglomeration of the asteroids, these substances /154
(under the influence of various energy sources) may have undergone destruction with subsequent reaction of the products.

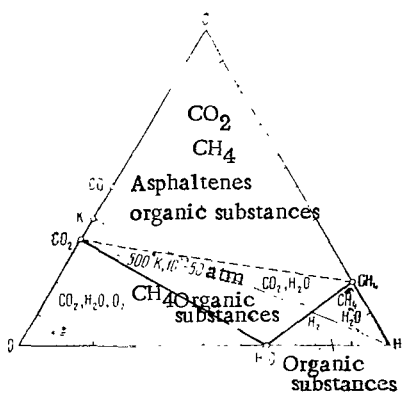

Figure 12. Phase Diagram of C-H-O. Regions Correspond to the Distribution of Products of Synthesis Under Different Conditions at the Early Stages of the Solar System. K -- cosmic ratio C:O.

Dayhoff et al. (1964) calculated the energy of formation of organic substances containing C, H, O from simple compounds. The phase diagram plotted on the basis of the calculated data under different conditions (Dayhoff et al., 1964) and confirmed experimentally (Studier et al., 1965, 1968) is shown on Figure 12.

During the development of the substance of the carbonaceous chondrites, the organic substances formed from simple original compounds underwent further changes. In particular, following the destruction of asteroidal bodies, they were subjected to cosmic and solar radiation. If they had undergone considerable changes at this time and had been subjected to destruction, we would expect to find in the carbonaceous chondrites with considerable cosmic age (meteorites of type III) a less carbonized carbonaceous substance. In reality, the degree

of carbonization decreases from type III meteorites to type I meteorites. However, these characteristics do not allow us to suggest that organic substances were formed from graphite in carbonaceous chondrites.

The mechanism of formation of complex organic compounds in carbonaceous chondrites in the process of degassing of volatile compounds in the course of agglomeration of asteroidal bodies, which was described briefly above, probably took place with the development of other bodies in the Solar System. It is felt that during the agglomeration of the substance forming the Earth the carbon was not in the form of CH_4 or CO_2, (it would have been lost during agglomeration), but in the form of carbides or organic substances with a condensed aromatic structure. In the course of differentiation of the substance of the Earth, these carbonaceous substances must have undergone destruction and been transported to the upper zones in the form of simple gaseous compounds.

The carbonaceous substances transported to the upper zones of the Earth's crust could have been synthesized here into complex organic compounds, on the base of which living matter could have developed with favorable conditions of the medium. /155

The results of a detailed study of the organic compounds in meteorites shows that these substances are similar to terrestrial organic compounds of biogenic origin, and to products of laboratory (and industrial) abiogenic synthesis of organic substances. This indicates that organic substances which are very sensitive to the conditions of existence have a similar nature of conversion regardless of where they are found (under terrestrial conditions or under the conditions on asteroidal bodies), and also independently of the biogenic or abiogenic nature of the initial carbonaceous substances. The regularity which was first mentioned by the author (Vdovykin, 1967, 1968) for exobiology is of primary importance.

Consequently, terrestrial organic compounds which gave rise to living matter may have been as complicated as the organic substances of the carbonaceous chondrites. The first microorganisms which appeared were heterotrophic. They may have used the complex organic substances which existed for

nourishment. It has been shown experimentally (Vdovykin, Pomortseva, 1962) that the organic compounds of carbonaceous chondrites may be used for the nourishment of microorganisms, supporting their growth. In the course of evolution of early microorganisms, there was a further development of photosynthetic forms of life, which had a critical influence on the changes in the composition of the Earth's atmosphere and on the characteristics of many geological processes (Vinogradov, 1967; Tugarinov, 1967). The most ancient deposits of remains of life forms in the rocks have an age of $2.5\text{-}3\cdot10^9$ years (Belsky et al., 1965, etc.), i.e., the transition from chemical evolution to biological evolution under terrestrial conditions took place almost 3.5 billion years ago. Even the early forms of life were able to accumulate the volatile isotope of carbon, C^{13}. For example, on the basis of the isotopic composition, the carbon of organic compounds of gold-uranium conglomerates from the Witwatersrand system in South Africa, the absolute age of which has been determined to be $2.15\cdot10^9$ years, there are $\delta\ C^{13}$ values between -2.24 and -3.28% (Hoffs, Schidlowski, 1967), in contrast to the isotopic composition of the carbon from the average meteoritic substance, which according to the authors' calculations is about - 2% $\sigma\ C^{13}$. In living systems, following their death and preservation in deposits, there are further changes in which the products of these conversions are sometimes very similar to the products of abiogenic synthesis of organic substances.

The similar nature of the behavior of carbonaceous compounds under different conditions, the existence of complex organic compounds in other heavenly bodies, especially meteorites, the considerable distribution of carbon and other "biophilic" elements in space -- all of these provide a /156
basis for considering that if there is life on other planets besides the Earth, it must be based, at least within the limits of the Solar System, on carbon, and has a protein nature. The conditions for the existence of protein forms of life are well known. Consequently, it is highly likely that life may be found on those planets of the Solar System whose physical and chemical conditions are favorable for maintaining protein forms of life. The studies of a number of planets that have been conducted in recent years with the aid of

spacecraft unfortunately do not indicate that the physical conditions on those planets might be favorable for the existence of such life forms.

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MICROBIOLOGICAL INVESTIGATIONS OF METEORITES

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ABSTRACT: Study of the penetration of microorganisms into rocks and meteorites under various climatic conditions. It is shown that no reliable results can be obtained from a search for extraterrestrial microorganisms in meteorites that were exposed to the ground for a prolonged period of time. Under any climatic conditions, the most important factor enhancing the penetration of microorganisms into meteorites is humidity. Some interesting results can only be obtained from meteorites that fell on stony ground, snow, or sand, and that were recovered shortly after the fall.

For many years, outstanding scientists have been suggesting that meteorites transport representatives of extraterrestrial life to Earth (Richter, 1865; Helmholtz, 1884; Thomson, 1871). However, all of these suggestions are of a purely speculative nature. In recent years, the study of space has again attracted the attention of scientists to this problem but in this case the impetus consisted of objective facts, established as the result of precise chemical analyses.

Undoubtedly the most interesting problem that arises in connection with the study of meteorites is the question of whether or not they contain traces of life. At the present time, the discussion between representatives of two opposite schools of thought is still incomplete, involving the origin of complex organic compounds which are found in meteorites.

The beginning of the dispute regarding the possibility of remnants of life in meteorites was triggered by Berzelius (1834) who was the first to find humus-like material in the Alais meteorite, which belongs to the group of carbonaceous chondrites, and suggested that this material was of a biological nature.

Later, many investigators (Woehler, 1858, 1859; Woehler, Hoernes, 1859; Smith, 1876; Roscoe, 1863; Berthelot, 1868) extracted organic compounds from this same Alais meteorite, as well as from the Cold Bokkveld and Orgey

carbonaceous chondrites. Using alcohol, ether and other solvents, the investigators successfully extracted organic substances from these meteorites.

In 1953, considerable attention was attracted by the work of Mueller (1953) who, after making extracts from the Cold Bokkeveld meteorite, claimed that about 25% of the organic substance of the meteorite was soluble in organic /158 solvents. Vdovykin (1960) performed a study of the organic substance extracted by organic solvents (without heating them) from the Migei, Staroye Boriskino, Groznaya and Cold Bokkeveld meteorites. The volume of these materials amounted to 0.07% of the weight of the meteorites, and they were made up of a complex mixture of compounds (hydrocarbons, humus-like substance, etc.).

Calvin and Vaughan (1960), as well as Briggs (1962) performed similar extractions with meteors of the same type -- Murray, Haripur and Mokoya -- using a Soxhlet apparatus and observed organic material amounting to 56 mg, 74 mg or 28 mg, respectively, per gram of meteor substance.

Nagy, Meinshein and Hennessy (1961), subjecting extracts and various fractions of the Orgey meteorite to mass-spectrometric analysis, found saturated hydrocarbons, which included normal paraffins, monocyclic, bicyclic and tricyclic; in terms of their molecular weight, they were similar to the hydrocarbons from modern marine sediments and grease.

An analysis of the results of the study of the composition of the properties of the organic substances in the carbonaceous chondrites will be found in a review article by Briggs and Mamikunian (1963) and in a monograph by Vdovykin (1967).

The abundance of organic materials in meteorites and their similarity with organic substances of terrestrial origin has stimulated many microbiologists to look for microorganisms in meteorites.

Pasteur was the first to subject meteorites to microbiological analysis. Using a special drill of his own design, he analyzed the internal parts of the Orgey meteorite and claimed a complete absence of microorganisms. Later, however, a number of reports appeared which indicated that microbes were

present in meteorites. Thus Galippe and Souffland (1921), after analyzing 21 meteorites of various types, found a great many mobile and immobile microorganisms growing on ordinary nutrient media. Somewhat later, Lipmann (1932, 1936), Roy (1935), Sisler (1961) and Bayriyev and Mamedov (1962) claimed that they had found microorganisms in various meteorites. In describing the method of microbiological investigation of meteorites, all of the authors emphasize that the surface of the meteorite was carefully sterilized prior to analysis and that all of the experiments were performed with observation of the necessary conditions of sterility.

However, the abundance of microorganisms that were found, their membership in the most diverse systematic groups of soil microorganisms and a number of considerations of a theoretical nature make these findings highly controversial. Even with the most careful sterilization of the surface of the meteorite to be analyzed, it is natural that their internal parts were /159
not sterilized. Samples were then collected from the central portions of the meteorite for microbiological analysis. As a matter of fact, this means that if the terrestrial microorganisms had penetrated into the internal parts of the meteorite, they would be detected on analysis. Therefore, it would be very difficult to say what the origin of the microorganisms that were found really was. In order to answer such claims, the authors, who found microbes (Lipmann, 1936; Claris, Nagy, 1961), point out that the small size of the pores in the meteorite and the position of the microbes in its central portions indicate that it is not possible for microorganisms to penetrate into the meteorite from outside.

To test these views experimentally, we performed a series of experiments with meteorites and rocks.

Experimental Section

Permeability of Meteorites and Rocks to Microorganisms

To establish the permeability of materials of different density to microorganisms, in the first series of experiments we studied rocks which differ in hardness and density.

TABLE 1. RESULTS OF MICROBIOLOGICAL ANALYSES OF STERILE ROCKS, STORED UNDER DIFFERENT CONDITIONS (CULTURED ON MEAT-PEPTONE BOUILLON)

/160

Sample and its origin	Storage Time	Samples Placed in Soil		Control samples stored in the Laboratory	
		Surface	Central Parts	Surface	Central Parts
Volcanic tuffs, Armenia, Arctic.	2 mos.	+	+	-	-
	9 mos.	+	+	-	-
	2 mos.	+	+	-	-
	1 mo.	+	+	-	-
	2 mos.	+	+	-	-
	1 mo.	+	+	-	-
	9 mos.	+	+	-	-
	2 mos.	+	+	-	-
	1 mo.	+	+	-	-
	3 weeks	+	+	-	-
	1 mo.	+	+	-	-
Pumice, Armenia, Ani.	2 mos.	+	+	-	-
	1 mo.	+	+	-	-
Talc; Eastern Siberia, Onot River.	1 mos.	+	+	-	-
	3 weeks	+	+	-	-
Magnesite; Armenia, Shordzha.	1 mo.	+	+	-	-
	3 weeks	+	+	-	-
Granite; Moscow Oblast'	9 mos.	+	+	-	-
Coal; Moscow Oblast'.	1 mo.	+	+	-	-

NOTE: + = presence of growth; - = absence of growth.

We studied the following rocks: tuffs, pumice, talc, magnesite, granite, and coal. Rock samples measuring 10 × 10 cm were heated for 8-12 hours in a muffle furnace at 400-500°. Then these samples were wrapped in parchment paper and sterilized again in an autoclave at 1 atm. Then some of the samples were removed from the paper and placed in a box with moist soil (two-thirds filled), while another part was stored in its wrapper under laboratory conditions. During the storage process, the rock samples were removed from the soil at different periods of time and subjected to microbiological analysis, with all necessary conditions of sterility being observed. At the same time, the samples stored under laboratory conditions were analyzed. The method of performing the microbiological analysis of the rocks and meteorites has been described in a previously published work (Imshenetskiy, Abyzov, 1966).

Samples collected from the central portions of the specimen were cultured on a meat-peptone bouillon. The results of these experiments are shown in Table 1.

The microbiological analyses of 19 tested samples of rock revealed that in the central parts of the previously sterilized sample, which was then placed in moist soil, soil microorganisms were always found. In the control samples, which were stored under laboratory conditions, we did not find any microorganisms at all. All of the tested samples were penetrable by microorganisms regardless of their density and hardness.

The second series of experiments was performed as follows. A vertical channel measuring 8-10 mm in diameter was drilled in the central portion of a sample of rock. Then this channel was plugged with a rubber stopper through which a glass tube had been pushed. The sample prepared in this fashion was sterilized several times in an autoclave and placed in a large glass vessel. The vessel was covered with a gauze covering, through which a glass tube was passed that was used for adding nutrient medium (meat-peptone bouillon). The glass vessel prepared in this fashion was sterilized in the autocalve together /161
with the sample. A general view of this vessel is shown in Figure 1. Then, using the glass tube inserted in the central channel that had been drilled in

the test sample, we added soil or pure culture of *Serratia marcescens*, grown on a liquid nutrient medium. Other samples which also had channels contained neither soil nor bacterial cultures. They served as the controls. All of the samples were placed in a thermostat. The data from these tests are shown in Tables 2 and 3.

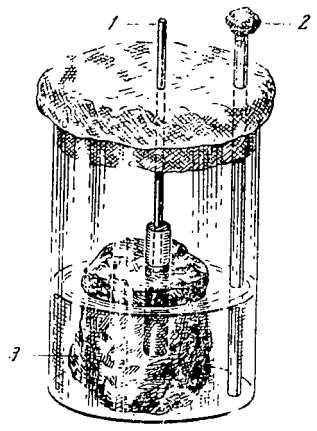

Figure 1. Apparatus:
1, Tube for adding soil or culture to the interior of the tested sample; 2, Tube for introducing nutrient medium, 3, Test sample.

The results of the experiments confirm the conclusions drawn earlier regarding the fact that all of the investigated samples of marble, pumice, limestone, tuff, talc, magnesite, sandstone and dunite are quite porous and permeable both to soil microorganisms as well as cells of *Serratia marcescens*. As early as the second day following the start of the experiment, excessive multiplication of microorganisms was observed in the bouillon surrounding the rock sample, while the control dishes with nutrient medium remained sterile. /162

TABLE 2. PENETRATION OF SOIL MICROFLORA THROUGH ROCK

Rock	Experiment						Control		
	I	II	III	IV	V	VI	I	II	III
Tuff	2	2	—	—	—	—	0	0	—
Limestone	2	—	—	—	—	—	0	—	—
Pumice	2	2	—	—	—	—	0	0	—
Marble	2	—	—	—	—	—	0	—	—
Talc	7	16	11	0	3	7	0	0	0

NOTE: The numbers correspond to the number of days required for noticeable clouding of the nutrient medium; 0 = absence of growth; — = absence of repeatability.

TABLE 3. PENETRATION OF CELLS OF *SERRATIA MARCESCENS* THROUGH ROCK

Rock	Experiment				Control		
	I	II	III	IV	I	II	III
Dunite	2	2	2	—	0	0	—
Magnesite	2	2	2	2	0	0	0
Sandstone	2	2	4	0	0	0	0

NOTE: The numbers correspond to the number of days required for significant turbidity of the nutrient bouillon; 0 = absence of growth; - = absence of repeatability.

TABLE 4. PERMEABILITY OF METEORITES FOR SOIL MICROORGANISMS

Meteorite	Experiment		Control	
	I	II	I	II
Sikhote-Alinskiy (iron octahedrite)	2	2	0	0
Chinge (iron ataxite)	0	-	0	-
Kunashak (stony chondrite)	8	-	0	-
Saratov (stony chondrite)	15	-	0	-

NOTE: The numbers correspond to the number of days required for significant turbidity of the meat-peptone bouillon surrounding the meteorite; - = absence of growth.

Hence, both series of tests showed that various rocks are readily permeable to microorganisms.

The third series of experiments were performed with meteorites. We studied the following meteorites: Sikhote-Alinskiy (iron octahedrite), Chinge (iron ataxite), Kunashak (stony chondrite), and Saratov (stony chondrite).

The permeability of these meteorites to microbes was tested in the same fashion as in the second series of experiments, i.e., by introducing soil into a central channel drilled in a meteorite. The meteorites were submerged in a meat-peptone bouillon.

The results of these experiments are shown in Table 4. These tables show that three meteorites were readily permeable to soil microorganisms. As early

as two days after being placed in the bouillon, extensive multiplication of microorganisms was observed around the Sikhote-Alinskiy meteorite. The controls were sterile. Negative results were obtained only in the experiment with the Chinge meteorite, which is an iron meteorite (ataxite), with a fine-grained structure, reminiscent of a metal alloy. Hence, the experiments which were performed indicate that all microbiological analyses of meteorites which have lain around for some time after falling on moist soil cannot be used to prove the presence of extraterrestrial microbes. Soil microflora together with the moisture of the soil penetrate quite rapidly into the central portions of the meteorite. Naturally, in this case careful sterilization of the surface of the meteorites and observation of all precautions during analyses are in vain. /163

Permeability of Meteorites and Rocks to Microorganisms Under Various Soil and Climate Conditions

It is possible for meteorites to fall at various points on the Earth's surface, under various soil and climate conditions and at different times of year. In this connection, it is necessary to study the contamination of meteorites by soil microorganisms, taking place in different types of soil and under various climatic conditions.

To solve this problem, the following three regions in the territory of the Soviet Union were selected because they differ sharply in terms of their soil and climate conditions:

1. The Turkmenian SSR. Stony soil in the Kopet-Daga Mountains (Firyuza) and sand from Karakum (Repetek station).

2. Moscow Oblast' (Solnechnogorskiy Rayon). Podzol cultivated soil.

3. Arctic, Dixon Island. Tundra frozen soil, covered with snow.

We studied samples of tuff, limestone, talc as well as meteorites. Samples measuring 10 x 10 cm, wrapped in parchment paper, were sterilized repeatedly in an autoclave at 1 atm. Microbiological analyses indicated complete sterility of these samples. A portion of the latter was kept closed under laboratory conditions. They served as the controls. Another portion of the sterilized samples was placed on exposed ground in these areas of the

Soviet Union. The samples were unwrapped before the start of the experiment. In the case of the stony ground and the frozen, snow covered soil, the samples were placed on the surface. In the case of the sand and the podzol, samples were buried for one-third of their length. The samples were unwrapped and placed on the ground with observation of all rules for asepsis. Each type of rock was tested twice. After the samples had been allowed to rest in the soil for a period of time that varied between four days and six months, the samples of rock were removed from the soil, the soil or snow that was still /164
clinging to them was carefully removed by wiping, and they were then wrapped in sterile paper and brought to the laboratory for microbiological analysis. Samples were taken from the surface of the rocks, from their peripheral layers and from their central portions. The quantitative analysis of the microorganisms was performed by the method of limiting dilutions in meat-peptone broth and the dish method using meat-peptone agar. For this purpose, we removed samples weighing 1 gram from the appropriate layers of the rock and then performed the dilutions. The number of microbes was counted in each gram of tested sample.

This method was used to perform tests twice in the Solnechnogorsk Rayon of Moscow Oblast': these tests took place in the autumn (November) and winter (at the end of January) of 1965. The results of these tests are shown in Table 5.

The microbiological analysis of the sterile samples of rock which were placed at various periods of time on exposed soil in Moscow Oblast' shows that soil microorganisms penetrate into the inner layers of rocks fully in the presence of a sufficient amount of moving moisture. Thus, in the central parts of the samples, which were placed in autumn on moist soil, we observed extensive microflora after only four days, while in the case of samples that were placed on frozen snow covered soil in winter the microbes did not even /165
penetrate to the depth of one cm in the course of 40 days.

TABLE 5. NUMBERS OF MICROBES IN VARIOUS LAYERS OF STERILE SAMPLES PLACED ON OPEN SOIL (MOSCOW OBLAST') AT DIFFERENT TIMES OF YEAR (IN THOUSANDS PER GRAM OF SUBSTANCE)

Sample Tested	Amount of Precipitation in the form of Rain, mm	Surface of Sample	1 cm from the Surface	Center of Sample
		Autumn, 4 days (November)		
Tuff	78.2	110,000	11,000	11,000
Limestone	78.2	110,000	7,000	2,500
Talc	78.2	110,000	250	700
		Winter, 40 days (from 29 January to 3 March)		
Tuff	0	25	0	0
Limestone	0	6	0	0
Talc	0	6	0	0
		Control (storage under laboratory conditions)		
Tuff	0	-	0	0
Limestone	0	-	0	0
Talc	0	-	0	0

According to the data from the Solnechnogorsk meteorological station, the average daily temperature on the surface of the soil during the period beginning on 29 January and ending on 9 March 1965 never went above zero degrees centigrade, which indicates an absence of mobile moisture.

On the stony soil in the Kopet-Daga Mountains (Firyuza) and on the Karakum sand, the sterile samples of rock were allowed to remain from April until October 1965, i.e., for a period of time when atmospheric precipitation is practically completely absent in this area. According to the data from the meteorological stations at Firyuza and Repetek, there was no atmospheric precipitation during the period indicated.

With no mobile moisture on the surface of the rocks, the internal layers of the samples are not invaded by soil microflora for a long period of time, regardless of the fact that microbes are always found on the surface of the sample. Thus, on the surfaces of samples of tuff, limestone and talc,

collected in the Kopet-Daga Mountains, we found 25, 13 and 6,000 cells per gram; at the centers of the samples and at a distance of 1 cm from the surface, no microorganisms were found. The same can be said of the samples which were placed in the Karakum sand; the difference lies in the fact that all three samples had the same number of microorganisms on their surfaces -- 110,000 per gram. Control samples that were stored in the laboratory remained sterile.

Similar tests were performed in the Arctic. The samples were placed on frozen snow covered ground on the territory of the Dixon Meteorological Station and kept there for five months. According to the data from the Dixon Meteorological Station, the average daily temperature on the surface of the soil during this period of time never rose above zero centigrade and consequently there was no mobile moisture.

In the Arctic, with no mobile moisture on the surface of the samples, the internal layers of the sterile rocks also remained completely free of microorganisms for a long period of time. On the surfaces of the samples of tuff, limestone and talc, we could always find microorganisms in amounts which were equal to 1.0, 2,5 and 0.6 thousand per gram of moist weight, respectively. During the same period of time, in the course of 100 days on the territory of the Dixon Meteorological Station, we measured the rate of contamination of sterile meteorites from Saratov and the Pervomayskiy settlement, which are classified as stony chondrites. As controls, we used sterile pieces of the same meteorites, which were wrapped in paper and stored in the laboratory. The results of the experiments revealed that microorganisms appeared on the surface of the meteorites in equal amounts -- 25,000 per gram of moist weight; the control samples in the central portions of the experimental meteorites remained sterile. We see that in the absence of mobile /166 moisture in the soil, the internal layers of meteorites may remain free of soil microflora for a long period of time, although rather large amounts of microorganisms are present on the surface of the meteorite.

Hence, these experiments demonstrate that meteorites that have fallen on dry stony soil, on snow in Arctic regions or on dry desert sand and are

collected immediately after they have fallen are most suitable for microbiological studies. As far as meteorites that have fallen in the middle latitudes are concerned, we are interested in using for microbiological investigations only those meteorites which have fallen during winter on snow or frozen soil and have been collected soon after they have fallen.

Conclusions

1. The presence of microbes of extraterrestrial origin in meteorites cannot be demonstrated by microbiological analysis of meteorites that have lain on damp soil.

2. In all of the soil and climate zones, the principal factor which promotes a rapid penetration of microorganisms into meteorites and rocks is soil moisture.

3. Meteorites that have fallen on dry stony soil or sand, as well as frozen ground or snow, and have been collected soon after they have fallen may be of interest for microbiological analysis.

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MODERN METHODS AND MEANS OF STERILIZATION OF SPACECRAFT

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ABSTRACT. The need to sterilize devices sent to other planets is discussed. Differences between methods used to sterilize spacecraft and those used in medicine are stressed. Four groups of sterilization methods are defined: hot, cold, combined and mechanical. Effects of dry, hot air, steam, ionizing radiation and UV radiation on physical substances are discussed. Bactericidal properties of certain chemicals and specially treated fabrics, paints, etc. are described.

As a result of the extensive study of space, the Soviet Union is able to send different types of stations and devices to the nearest planets for purposes of extensive investigation of the planets and for seeking life on them. To solve the latter question, it is necessary first of all to prevent the transport of terrestrial microorganisms to the nearby planets and into the space surrounding those planets. Various types of preventive measures have been provided for doing this (Sneath, 1961; Atwood, 1966).

It is also necessary to prevent the possible transport of life from other planets to Earth, since we cannot exclude the possibility that microscopic living substances exist on other planets which could be very dangerous for the inhabitants of Earth (Cooley, Schalkowsky, 1966; Farmer, 1966).

At the present time, more and more attention is being devoted to ways of sterilizing spacecraft to be sent to other planets and designed to return from other planets to Earth (Nicks, Reynolds, 1963). In seeking methods and devising ways of sterilizing spacecraft, researchers are proceeding mainly on the basis of the extensive data available in the literature on the topic of disinfection and sterilization, as well as the practical experience in the use of various substances used for this purpose in the national economy.

Sterilization is widely employed in medicine, especially surgery, in the food industry for products which are to be stored, and other branches of the national economy. This means that at the present time a great many different devices and methods of sterilization have been considerably developed (Horowitz, 1966).

The famous English surgeon Lister was the first (in 1867) to publish his studies on the use of antiseptic substances in surgery. The Russian School of Surgery led by Pirogov increased and expanded the data in regard to this /168
matter. By the 1880's, methods of antisepsis and asepsis were widely known. The essence of the method of asepsis in surgery boiled down to the following: nothing which has not been sterilized may come in contact with a wound. This principle of surgery is maintained at the present time.

As a result, surgery has made much greater strides than it did during the entire time of its existence prior to 1867.

Finally, not all of the known methods and means are suitable for sterilization of spacecraft. A spacecraft must not contain any terrestrial microorganisms of any kind, including those that live in the soil A great many of the materials that are used in the construction of a spacecraft will not stand exposure to the most widely used methods and means of sterilization, especially the action of high temperatures, etc.

The methods of sterilization that are used at the present time can be divided into three groups: heat, cold and a combination of the two.

The above methods use a great many different substances. Almost all of the existing methods and means are used to one degree or another in the sterilization of spacecraft. However, none of the existing methods or means can ensure sterility of the spacecraft by itself. Further detailed studies are necessary to improve the methods of sterilization of the specific materials that go to make up a spacecraft and its models. The effectiveness of using a particular method and the substances used for sterilization depend to a large degree on the volume, configuration and physical characteristics of the objects to be sterilized (Miles, 1966).

As far as physical agents are concerned, most extensive use is made of steam and dry hot air, while ionizing radiation has come into use recently. Steam is the most reliable sterilizing agent but it is not very good for sterilization of spacecraft for two reasons: many parts and assemblies of spacecraft cannot withstand temperatures of 100° and high humidity.

Of the other most reliable methods of sterilization, dry hot air is worthy of attention; it is widely used in microbiological practice, heated to 160-180° with exposures of 60 to 30 minutes.

Hot air penetrates well into all parts of the apparatus and the heating is easily regulated. However, this agent, in the temperature and exposure ranges at which it is widely employed in medical and microbiological practice, is not very suitable for sterilization of spacecraft because many materials that are used in the assembly of the latter cannot withstand temperatures of 160°. Consequently, dry hot air at such temperatures cannot be recommended for sterilization of spacecraft. In this connection, studies are going /169
forward to determine the possibility of using temperatures lower than 160° for the sterilization of spacecraft.

In the USSR and USA, considerable attention has been devoted to studying the possibility of using dry hot air (Koesterer, 1964, 1965).

The work is divided into two parts: first of all, the possibility of using hot air (105-150°) is being studied; secondly, materials are selected for use in the construction of spacecraft which are able to withstand heating to 135-150° (Bruch et al., 1962).

It has been found that heating different types of objects to 149° for 40 minutes ensures sterility. In an atmosphere of dry nitrogen, at a temperature of 145°, the exposure time was 35 hours. However, heat sterilization at 145° for 36 hours has an unfavorable effect on the majority of sensitive photocathodes. They contain cesium, which liquefies at 145°. The designers must work on the development of bialkaline photocathodes. The possibility of sterilization at lower temperatures (for example, 105°) has been demonstrated, but the exposure time then increases to 14 days. The construction of the

spacecraft which were sent to Venus, however, necessitated the use of thermo-resistant materials, since the temperature on Venus is above 100°.

The next method which might be used for sterilization of individual parts is ionizing radiation, particularly gamma rays, beta particles and relatively heavy neutrons, protons, etc. At the present time, ionizing radiation is widely used for sterilization in many countries of the world. Numerous investigations of the possibility of using ionizing radiation are underway in the USSR.

The sensitivity of living matter to ionizing radiation differs. A man may die as a result of exposure to 600 rads; some microorganisms die when exposed to 100,000 rads; millions of rads (2.5-5 Mrad) and required to kill the spores of microorganisms or viruses.

Of the microorganisms that have been studied, the most resistant to radiation are those of *Clostridium botulinum*, which causes food poisoning and can be killed by doses of 2.5-4 Mrad. Data are available indicating that the virus of smallpox vacine, dried at negative temperatures, will die at still higher doses.

The level of the sterilizing dose depends both on the nature of the material subjected to sterilization and the number of microorganisms that are present on the material being irradiated. In this connection, when using highly contaminated objects for irradiation, the exposure time is increased in comparison to that for objects which are slightly contaminated by micro-organisms.

In sterilizing spacecraft, an effort is made to kill all forms of micro- /170
organisms, so that we can recommend only high sterilizing doses of ionizing radiation; in addition, many of the materials which are used for the assembly of spacecraft will not withstand doses as high as 5 Mrad. Hence, ionizing radiation may be used for sterilization of only a few types of materials.

As far as physical agents are concerned, we must consider ultraviolet rays with a wavelength of 2537 Å. Many countries are conducting research into the bactericidal properties of ultraviolet rays. At the present time, Soviet

industry produces six types of ultraviolet apparatus; wall-mounted and ceiling lamps are completely suitable for irradiation of the surface of materials for purposes of reducing the contamination of the parts of an assembled spacecraft. This type of lamp is switched on when the laboratory is not at work. During work times in the laboratory, reflected ultraviolet rays may be used. The technique of using ultraviolet lights has been quite well developed. Other physical agents, for example ultrasound or currents of ultrasonic frequency, in our opinion, cannot be recommended for sterilization in conjunction with a number of shortcomings to which they are prone.

In sterilizing spacecraft, various chemical substances can be used both in solution and in the form of gases. Among the gaseous chemical compounds, the ones most worthy of our attention are ethylene oxide and methyl bromide which can be used for sterilization both of individual parts of a spacecraft and for the spacecraft as a whole.

Ethylene oxide possesses high bactericidal, viricidal, fungicidal insecticidal and ovicidal properties. In many countries, ethylene oxide is used in both the pure form (in France) and mixed with other gases. It is capable of penetrating different types of cloth (30 layers) and materials. Hot cloth, contaminated with bacterial spores and placed in paper sealed covers, can be decontaminated by ethylene oxide at a concentration of 450 mg/l at room temperature in 4-6 hours. Ethylene oxide is adsorbed by the material to be sterilized. Desorbtion of its principal mass lasts 1.5-2 hours, while small amounts remain for 4 or more days. In connection with the fact that ethylene oxide in the pure form is inflammable and liable to explode in concentrations of 3 to 80% in air, it is used in conjunction with substances which will not support combustion. A mixture of ethylene oxide and carbon dioxide is the one most used abroad, while recently it has been mixed with freon as well. When used in the pure form, a vacuum is produced in the chamber. A mixture containing 10% ethylene oxide and 90% carbon dioxide is /171
the one most widely employed. This mixture is known under the name of cartox or carboxide. In the USSR and USA, the possibility of using ethylene oxide for sterilizing spacecraft has been studied at different temperatures -- from

18 to 40° -- and for different exposure times (up to 24 hours). Positive results have been obtained.

A number of similar substances are known: oxifum, which consists of 80% carbon dioxide and 20% ethylene oxide (it can ignite if the ethylene oxide concentration is 19-40%); cryoxide a, which contains 11% ethylene oxide, 79% trichlorofluoromethane and 10% dichlorodifluoromethane; cryoxide b, which consists of 11% ethylene oxide, 54% trichlorofluoromethane and 35% dichlorodifluoromethane, and penoxide, which contains 12% ethylene oxide and 88% dichlorodifluoromethane. When using ethylene oxide mixed with the above compounds, increased pressure is always developed in the chamber and this requires a complex and costly apparatus. The sterilizing effect of a mixture of ethylene oxide with various inert gases, especially freon, is weaker than that of a mixture of ethylene oxide with carbon dioxide. In the gaseous state, this type of mixture does not harm the cloth, paper, documents, polished and painted furniture and metal; it does not alter the strength of plastics.

In the USA, particular attention is being devoted to mixtures containing 88% freon and 12% ethylene oxide, with 500 mg/liter of this mixture at a relative humidity of 35% and temperatures of 24 and 40°, exposure time = 24 hours. According to the data of Phillips (1966), it is easy to sterilize using a gas containing 12% by weight of ethylene oxide and 88% freon 12.

In contrast to foreign methods of using ethylene oxide, Prishchep in the USSR (1964) with his associates (1967), Osipyan and the author of this article (1967) have developed a method of sterilizing spacecraft by using a mixture of ethylene oxide and methyl bromide ("OB" mixture). Methyl bromide can be used in the pure form, but it is not practical, since the methyl bromide is much inferior to ethylene oxide in terms of its bactericidal properties. A mixture of the two bactericidal gases is very effective. In addition, methyl bromide will not support combustion. In working out ways of sterilization using the "OB" mixture, the effect of gas sterilization on many materials was studied: painted surfaces, adhesives, lubricants, resins,

adhesive compounds and radioelements, used for the manufacture of spacecraft. A great many different materials and devices were tested, but this of course was insufficient. The study of materials and coatings following sterilization was performed in accordance with existing methods of testing the strength and durability of materials. Five samples of each material were subjected to the action of the gas mixture and five samples serve as controls. The results of the testing of the experimental and control samples revealed that sterilization with the "OB" mixture has no effect on the physical and chemical properties of the materials and does not change the external appearance of the materials /172 or coatings.

The dielectric characteristics of the wires which were used in the experiment (4 types) did not change. We studied the effect of gas sterilization on coatings, radioelements, as well as capacitors, diodes, triodes, resistors, relays, connectors, remote-control switches, etc. A test of the radioelements before and after sterilization as well as after one and three months of storage showed that the electrical parameters of the radioelements did not change and remained within the limits set by the technical conditions. Variations between the results of measurements of the parameters at various stages of the tests in the case of the sterilized samples of radioelements were of the same order as those among the controls which had not been sterilized.

A considerable amount of work was done in studying the effect of gas sterilization using the "OB" mixture on plastics and resins, used in medicine (Shcheglova, Gerbova, 1965). A test of five different samples of plastic and parts made of them showed that sterilization has no effect on their physical and mechanical properties, with the exception of polystyroene microcracks appeared in Petri dishes made of this material after sterilization. All of these data indicate that the gas method may be used for sterilization of spacecraft.

When sterilizing liquids which are aboard the spacecraft, especially oil, it is possible to use a mechanical method of sterilization -- filtration. However, in order to get rid of viruses, special filters must be used.

The bactericidal substances in the form of solutions possess higher effectiveness than when in the gaseous state, but solutions are not suitable for sterilization of the entire spacecraft. They can only be recommended for sterilizing individual parts during the assembly of a spacecraft. Chemical substances in solutions may be used for sanitary and hygienic packing and cleaning of the laboratories where the assembly of the spacecraft takes place, for reducing the content of microflora growth in the environment and on the parts of the object being assembled. This will lead to a decrease in the number of microorganisms and have a positive influence on subsequent sterilization of the assembled objects.

Success in sterilization of an entire spacecraft depends to a considerable degree on the level of initial contamination of the parts. Therefore, in order to prevent the accumulation of microorganisms in the laboratories and on the parts of the apparatus, especially in the assembly laboratory, it is recommended that strict sanitary and hygienic requirements be imposed (cleaning of the area, filtration of the air that enters the laboratory, careful wiping of the equipment, observation of hygiene and a strict work regime by the personnel).

Various chemical substances used for the treatment of different surfaces /173
in industrial environments have been studied. One of the best has been found to be hydrogen peroxide, which (as we know), possesses bactericidal, sporicidal and viricidal properties. Concentrated solutions of hydrogen peroxide are good oxidizers; the hydrogen peroxide breaks down completely in the course of oxidation, and does not generate any side products. Experiments performed under laboratory conditions have shown that hydrogen peroxide is effective with respect to microorganisms that are in the vegetative state in a concentration of 3-5%, and with regard to spores -- in a concentration of 3-10% (depending on the type of spores); concentrations of 1 to 5% are needed to kill viruses. A disadvantage of hydrogen peroxide is the high surface tension (73 dynes/cm); however, this can be reduced by adding surfactants, for example, 0.5% sulfanol. To give the hydrogen peroxide solution cleaning power, preparations have been selected from the group of anionactive detergents, with which it is compatible in any ratio. The addition of such anionactive

substances to hydrogen peroxide reduces the surface tension of the solution to 28.8 dynes/cm, which in turn makes it possible to lower the standard for consumption of peroxide and simultaneously ensure complete and uniform coating of the surface. A mixture of hydrogen peroxide with a cleaning substance will then have bactericidal and sporicidal activity (Shumayeva, 1966); treatment of surfaces infected with vegetative and sporogenous forms of microorganisms, using the method of wiping and using 70-100 ml/m^2, will ensure reducing contamination by 99.99-100% with a single wipe of the surface of the tile or glass, while two wipes are necessary for wood. Hydrogen peroxide containing a cleaning agent will not alter the structure of wooden painted or unpainted surfaces, plastic and polymer materials, dense resins and many metals. It may also be used for decontaminating dropcloths and special clothing. Increasing the temperature reinforces the activity of this mixture with respect to microorganisms. Thus, for example, if the sterility of various objects (including plastics and resins) contaminated with vegetative forms of microorganisms is established at a temperature of the solution of 20-22° by submerging the object in a 3% solution of hydrogen peroxide with 0.5% detergent, while objects infected with spores of malignant anthrax can be decontaminated by immersing them in a 6% solution of this mixture, increasing the temperature of the solutions to 50° means that the concentration of hydrogen peroxide can be reduced by a factor of two. Hence, using solutions of hydrogen peroxide mixed with anionactive detergents provides a wide range of effectiveness and reliable performance in treating surfaces and the air of the environment.

There are many other bactericides which possess sporicidal properties, but all of them (for one reason or another) cannot be recommended for these purposes, since some of them have an unpleasant odor while others corrode /174
metals, etc. One such preparation is beta-propiolactone, a 2% solution of which may be used for sterilization. In the USA, it is recommended for sterilization of individual parts of a spacecraft.

From the preceding brief survey of the results of investigations, it follows that the problem of sterilization of spacecraft requires further research. The difficulty lies in the fact that all of the substances, in

addition to prolonged exposure at high temperature and ionizing radiation at very high doses, still cannot penetrate all of the parts of a spacecraft, especially the areas where parts are fastened or glued on, etc. Hence, all measures which reduce the contamination of the spacecraft must be used.

Among the measures which are used in the sterile assembly of apparatus, we may include the use of biologically active materials. It is desireable to use those materials which have a high bactericidal action and sporicidal effectiveness with a wide range of antimicrobial action. Recently, many investigators have been working on the development and study of various materials: cloth, rubber, lacquer-film coatings, which have antimicrobial characteristics. Antimicrobial properties have been conferred on materials by means of impregnation or chemical combinations of bactericides with macromolecular polymers (Shcheglova, Gerbova, 1965; Virnik, Mal'tsev, 1966). Advances in chemistry during the last decade have made it possible to obtain materials whose composition includes biologically active groupings, held together by chemical bonds of three types: covalant, coordinate and ionic.

In particular, cellulose cloth was studied, as well as cloth made of polyvinyl fiber and capron. The following preparations were used to confer antimicrobial properties on these types of cloth: salts of heavy metals, antibiotics, quaternary-ammonium compounds and preparations in the phenol series. Cellulose and synthetic fabrics containing these preparations have high antimicrobial activity: their contamination with *Staphyllococcus aureus* and intestinal bacilli is reduced by 82-100%; the ability of spores of *Bacillus anthracoides* is reduced by 75-98% when a 30-60 minute exposure is given.

Combining preparations with cloth by means of chemical bonds has considerable advantages over the impregnation of the materials with the same preparations. The antimicrobial activity is retained completely or insignificantly reduced even after 20-50 washings. Low (-14°) and high (+120°) temperatures for 30 minutes and prolonged storage for 2-3 years (under room conditions) have no effect on the initial antimicrobial activity of the cloth containing the above mentioned preparations. Antimicrobial cloth is not toxic for warm-blooded animals. /175

Objects of personal hygiene, made of cloth containing 6% hexachlorophene or 20% 5-nitrofuryl-2-acrolein, tested in long (15-30 days) experiments, showed less contamination (from 2 to 130 times) than the controls. Cloth with hexachlorophene possessed higher antimicrobial activity than cloth containing 5-nitrofuryl-2-acrolein. Antimicrobial underclothing had no harmful effect on the skin and organisms of man.

Rubber with antimicrobial properties is also of considerable interest; it can be obtained by adding bactericides to the rubber mixture. Compounds of the phenol series hold the most promise. These preparations are relatively resistant to the action of external factors and have active bactericidal properties. Rubber based on natural and artificial latex, containing 3-5% parts by weight of pentachlorophenol, phenol, oxidaphenyl, etc., has a high bactericidal activity. Mortality of *Staphylococcus aureus* and intestinal bacilli is 92-100%, while that among spores of anthracoid is 70-98%. The use of similar amounts of bactericidal preparations causes no deterioration of the physical and mechanical characteristics of the rubber. The preparations are firmly held in the rubber. Long continuous exposure of the samples to water (for 30 days) and the action of high temperature (80-100°) for three days under conditions of artificial aging causes no desorption of the included preparation, and the bactericidal properties remain at the same level. Rubber that is made from natural latex has much higher antimicrobial activity than rubber that is made from artificial latex. The antimicrobial cloth and types of rubber described above have no toxic effect as far as warm-blooded animals are concerned.

In addition, the development of self-sterilizing paints and films, adhesives and other materials is very important; these can be used for painting diverse parts of a spacecraft. Coatings of this kind would be able to ensure absence of microflora not only on the surface of the instruments aboard the spacecraft but also beneath the coatings of paint. Thus, silicate bactericidal paint can be used. In addition to the materials listed above, efforts are being made to make self-sterilizing plastics. Biologically active materials, especially paint coatings and various types of rubber, obviously could be used in building spacecraft, and this would ensure a considerable

reduction in the microbial contamination of the assemblies and parts of the spacecraft.

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THE PHYSIOLOGY OF GROWTH OF SPORES OF *BACILLUS STEAROTHERMOPHILUS*

V. I. Seregin

ABSTRACT: Spore germination is stimulated by preheating to 100°C for 10 min. An amino acid concentration of 0.01 mol in an L-arginine medium is optimal for germination. An increase in carbohydrate concentration from 0.001 to 0.1 mol results in a rise of the germination percentage; trehalose in 0.01 mol concentration is most effective.

As we know, preliminary heating of a suspension of spores promotes their growth; at different temperatures and heating times, the number of spores which grow will vary (Hyatt, Levinson, 1960; Cook, Brown, 1965). In addition, the concentration of various "initiators" is very important for the growth of spores (Filds, Finley, 1955; Hermier, 1962a, b). Vitamins of the B group and /177 other substances act as "initiators" for spore growth (O'Brien, Campbell, 1957).

The purpose of this work was to investigate further the conditions for the growth of spores of *Bacillus stearothermophilus*, strain 214 (Seregin, 1968 a, b).

Experimental Section

Method

The object of the investigation was a culture of *B. stearothermophilus*, strain 214. In order to obtain the spores, a two-day culture which was grown on tilted potato agar at 55° was transferred to tomato agar (Amaha et al., 1956), consisting of 10% tomato juice, 0.5% KH_2PO_4, and 2% agar. After culturing for seven days at 55°, there was a nearly 10% spore formation. Immediately prior to the experiment, a suspension of the spores was centrifuged according to the method of Wolf and Mahmoud (1957).

To begin with, we studied the effect of prolonged thermal activation on the growth of spores in the experimental culture. To do this, the spores which had been rinsed with sterile water were activated by heating them for

5, 10, 15, 20, 25 and 30 minutes at 100°. The activated spores were placed on a medium with the following composition (in g/l of distilled water): K_2HPO_4 -- 30%; KH_2PO_4 -- 10%; NH_4Cl -- 5%; Na_2SO_4 -- 1%; $MgSO_4 \cdot 7H_2O$ -- 0.1%; $MnSO_4$ -- 0.01%; $FeSO_4 \cdot 7H_2O$ -- 0.01%; $CaCl_2$ -- 0.001%; yeast extract (Difco) -- 0.1% (by volume), pH 7.3. As a control, we used a volume of growing spores that had not been activated by heating, in a medium of the same composition. The initial concentration of spores in the medium was $7.48 \cdot 10^6$ per ml.

In the next series of experiments, we studied the dependence of the growth of the spores on the concentration in the medium of amino acids, carbohydrates and certain of their derivatives. We added these substances to the mineral medium without yeast extract in concentrations of 0.001, 0.01 and 0.1 M; the experiments were performed in the medium with 0.001 M amino acids in the presence of trehalose. In the first control, the medium did not contain trehalose while in the second there was no trehalose, no amino acids, no carbohydrates or their derivatives. The concentration of spores in both cases was $6.9 \cdot 10^6$ per ml.

In other experiments, we studied the growth of spores in a medium which contained certain vitamins. A complex of vitamins including thiamine (1,000 γ/l), pantothenic and nicotinic acids -- 1,000 γ/l, riboflavin -- 200 γ/l, inosite -- 30 γ/l and cobalamine -- 2 γ/l was added to the above mentioned mineral medium with L-arginine (0.01 M) and trehalose (0.01 M). In addition, with subsequent exclusion of vitamins from this complex, we studied the growth of spores. In the control, we found a relative content of growing spores without addition of vitamins. The original concentration of the spores /178
in the medium was equal to $7.5 \cdot 10^6$ γ/ml.

Later, we studied the growth of spores in a medium containing substances which are most effective as far as growth is concerned. The percentage of the growth of the spores in this medium was compared in a medium of this kind and in a standard medium with thioglycolate of the following composition (in g/l of distilled water): acid-pancratic hydrolysate of protein -- 15; yeast extract -- 5; NaCl -- 2.5; Na_2HPO_4 -- 2.5; agar-agar -- 1; glucose -- 5; cystein -- 0.75; sodium thioglycolate -- 0.3; pH -- 7.0. The number of spores was equal to $7.2 \cdot 10^6$ per ml.

To determine the number of growing spores, we used the change in their optical characteristics in the course of growth. The culture was grown in Erlenmeyer flasks (capacity of 250 ml, with 50 ml of substrate). Using a Goryeyev chamber (1 ml of fluid), we counted the original number of spores, which was taken as 100%, and the number of spores which did not grow following 24 hours exposure at 55°. The percentage of growth was determined by the number of the latter.

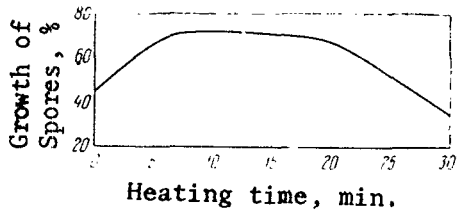

Figure 1. Effect of Duration of Heat Treatment of Spores of *Bacillus stearothermophilus* on their growth.

Dependence of Spore Growth on Duration of Thermal Activation

Figure 1 shows data on the growth of spores as a function of the duration of heat treatment. The growth curve was plotted using points that were obtained from six determinations. These data indicate that a mineral medium with yeast extract (control) shows a growth of 45% of the spores that were not activated by heating. Preliminary heat treatment of a spore suspension for 5 and 10 minutes increases growth by 69 and 72%, respectively. With further heating (10, 15 and 20 minutes), there is a more or less stationary fraction which decreases gradually (heating for 25 minutes), followed by a sharp drop in the amount of spores which grow (36%) with heating for 30 minutes.

Effect of Amino Acids, Carbohydrates and Certain of their Derivatives on the Growth of Spores /179

The dependence of the growth of spores of *Bacillus stearothermophilus* on the concentration of substances added to the nutrient medium is shown in the table. The results of the experiments indicate that when the concentration of amino acids is raised from 0.001 to 0.01 M the percentage of growth of spores increases reaching a maximum in a medium with L-aspartic acid (57%) and L-arginine (54%).

INFLUENCE OF VARIOUS CONCENTRATIONS OF AMINO ACIDS, CARBOHYDRATES AND CERTAIN OF THEIR DERIVATIVES ON THE GROWTH OF SPORES OF *BACILLUS STEAROTHERMOPHILUS*, STRAIN 214

Composition of Nutrient Medium	Concentration of Amino Acids		
	0.001 M	0.01 M	0.1 M
Medium with trehalose, 0.001 M (control)	19	--	--
Ditto, +			
L-arginine	23	54	20
L-aspartic acid	17	57	33
L-glutaminic acid	18	48	39
L-ornithine	20	44	30
L-lysine	17	42	30
L-proline	17	47	27
DL-treonine	4	13	10
L-leucine	6	19	21
DL-isoleucine	3	20	10
L-histidine HCl	2	41	25
L-valine	6	28	40
Medium without trehalose (control)	--	--	--
Ditto, +			
Trehalose	21	60	67
Glucose	17	39	42
Maltose	29	47	54
Saccharose	10	46	51
Galactose	20	42	44
Lactose	17	55	64
Sorbose	14	34	37
Arabinose	36	34	32
Glucosamine	34	44	54
Mannite	27	33	41

NOTE: The growth of spores as expressed in percentages relative to the original number of spores. The medium without trehalose (control) contained 3% of spores which grew.

The percentage of growth of spores decreases quite significantly with an /180
increase in the concentration of the majority of amino acids in the medium, with the exception of L-valine and L-leucine, where there is a further increase in the number of spores which grow to 40 and 21%, respectively. It is striking that in the medium with a concentration of certain amino acids equal to 0.001 M (DL-treonine, L-leucine, DL-isoleuciné, L-histidine HCl and L-valine) there is a very low degree of growth (4% on the average), i.e.,

much lower than in the control (19%), while in a medium containing other amino acids (L-arginine, L-ornithine, L-glutaminic and L-aspartic acids, L-lysine and L-proline), at the same concentration, 18% of the spores on the average grew, i.e., almost the same amount as in the control.

Similar results are observed at a concentration of amino acids equal to 0.01 M. In a medium containing DL-treonine, L-leucine, DL-isoleucine, and L-valine, 20% of the spores on the average grew, while when other amino acids were added (L-arginine, L-aspartic and L-glutaminic acid, L-ornithine, L-lysine, L-proline), the number of spores which grew amounted to 47% on the average. With a further increase in the concentration in the medium of DL-treonine, DL-isoleucine, L-histidine the growth of the spores was reduced to 17% and to 30% in a medium with other amino acids.

As far as the effect of carbohydrates on the growth of spores is concerned, the data presented in Table 1 indicate that as the concentration of nearly all of the carbohydrates increases, the number of spores that grow likewise increases, reaching a maximum in a medium with 0.01 M trehalose (67%). The only exception is arabinose. In a concentration of 0.001 M, it is most favorable for growth (36% of the spores grew). In other concentrations (0.01 and 0.1 M) the number of spores which grew amounted to 34 and 32%, respectively.

After the experiment with the carbohydrates, the increase in the concentration in a mineral medium of certain of their derivatives (glucosamine, manite) leads to an increase in the percentage of spore growth. In addition, it is also necessary to point out that manite turned out to be more effective than glucosamine in a concentration of 0.001 M. In other concentrations, the percentage of growth was higher in a medium containing glucosamine.

The Effect of Certain Vitamins on the Growth of Spores of the Investigated Culture

The results which indicate the growth of spores of *Bacillus stearothermophilus* in a medium containing certain vitamins are presented on page 225. In a medium with a complex of investigated vitamins, the number of spores which grow was 12% higher than in the control. With subsequent exclusion of

pantothenic and nicotinic acids from this complex, as well as riboflavine, /181
the effectiveness of the growth changed insignificantly and was 66% on the average. When other vitamines were missing from the medium (thiamine, inosite, cobalamine), the degree of growth of spores decreased to 54% during the first two cases and 46% in the last, i.e., the number of spores which grew was less than in the control (57%). This indicates the fact that the spores of this culture require thiamine, inosite and especially cobalamine in order to grow.

Composition of Nutrient Medium	Concentration of Vitamins, γ/l	Growth of Spores, %
Medium with L-arginine, 0.01 M and tregalose, 0.01 M.	--	57
Ditto plus complex of vitamins	--	69
Ditto, without:		
Thiamine	1,000	54
Pantothenic acid	1,000	65
Nicotinic acid	1,000	66
Riboflavine	200	67
Inosite	30	54
Cobalamine	2	46

Growth of Spores in Selected and Standard Media

We demonstrated earlier that yeast extract, trehalose (Seregin, 1968 a,b) and cobalamine are most favorable for the growth of spores. These substances were added to a mineral medium in concentrations of 0.1%, 0.01 M and 2 γ/l, respectively. Growth of spores in this medium was the same as the growth in the standard medium. The results of spore growth in these media are shown in Figure 2. The curves showing growth indicate that the percentage of spores which grow in the selected medium in the course of 24 hours incubation is significantly higher than in the standard medium. If we look at the percentage of spores which grew in the individual time intervals, we can see that 2 hours spent in the selected medium resulted in growth of the same number of spores as in the standard medium after 24 hours.

Evaluation of the Results

Many authors have pointed out that heating spores has a positive effect on their growth (Curran, Evans, 1944, 1945; Reynolds, Lichtenstein, 1949; Powell, Hunter, 1955; Treadwell et al., 1958). In addition, Cook and Brown (1965) demonstrated in their work a similar effect of heat treatment on the growth of the spores of *Bacillus stearothermophilus*. These data confirm the results of our work and it follows that heating a suspension of spores to 100° for 10 minutes is most favorable for the growth of the spores in the /182
experimental culture. Obviously, this procedure is optimal for the activation of the enzyme system of spore growth. It was shown in the works of Falcone and Coraco (1958, 1960) that heat treatment affects the fermentative apparatus of the spores. It was also found that as a result of heating there is a splitting of the L-alanine, the DNA-Ca^{++} complex and other substances (Levinson, Hyatt, 1955; Harrell, Martini, 1957). On the basis of these data, Levinson and Hyatt (1955) suggested that heat treatment acts as the principal metabolic factor which results in the endogenic L-alanine being liberated in the free state, which in turn leads to a rise in the catalytic activity of the spore enzymes.

Many papers mention different effectiveness of growth of spores of various species of Bacillus depending on the concentration of amino acids, carbohydrates and other substances in the medium (Finley, 1955; Hermier, 1962 a, b; Martin, Harper, 1963). They reported that individual substances in small concentrations stimulate the growth of spores of some forms of bacteria, while they are ineffective as far as spores of other species are concerned, even in large concentrations. In addition, there are considerable differences in the growth of spores of strains of certain bacteria under the influence of the same substances.

In our work we also noticed a considerable diversity in the action of amino acids, carbohydrates and other substances as far as the growth of spores is concerned. This diversity also applies to the concentration of substances in the growth medium, which complicates the observation of the requirements of the spores in the experimental culture during their growth, although it could

be followed in some cases. For example, the increase in the concentration of carbohydrates in the medium leads to an increase in the number of spores which grow. This is not observed with an increase in the concentration in the majority of amino acids.

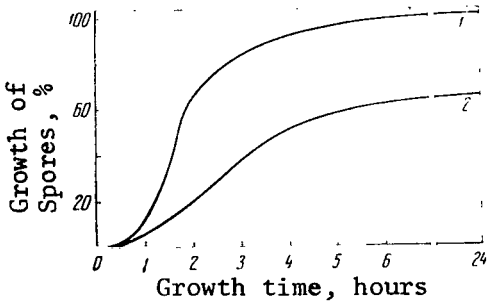

Figure 2. Growth of Spores of *Bacillus stearothermophilus* in the Selected and Standard Media.
Before the experiment, the spores were activated by heating them at 100° for 10 minutes, after which they were added at a rate of $1.2 \cdot 10^6$ to 1 ml to the medium, SS -- calculation of the percentage of growth in the interval from 6 to 24 hours. 1, Growth in selected medium; 2, Growth in standard medium.

Strain differences in the growth of spores have also been detected in media containing certain vitamins. Thus, it was established in the work of O'Brien and Campbell (1957) that nicotinic acid is necessary for growth of spores of *B. stearothermophilus*, while in our work we found that nicotinic acid has an insignificant effect on the growth of spores of the investigated strain (214) of this same species. Other vitamins (e.g., thiamine, inosite and especially cobalamine), on the other hand, are necessary for growth. It is interesting that cobalamine in small concentrations (2 γ/l) has a greater effect on the growth of spores than other vitamins in higher concentrations. /183

Diversity in the growth of the spores of the investigated culture obviously has to do with different permeability of the substances to the spores or the degree of readiness of the spores to metabolize a specific substrate, or is related to the stimulation or suppression of the growth by substances in various concentrations (Wolf, 1961). Obviously, we are dealing here with the complex action of factors which combine to stimulate the growth or cause its partial inhibition.

In conclusion, we should point out that the study of the growth of spores in a selected medium shows how rapidly growth takes place in it in comparison to a standard medium. This considerably expands the possibilities of checking the sterility of objects. Finally, the growth of spores is influenced by various physical and chemical factors whose study should be the object of further study.

Hence, we established as a result of our work that the optimum medium for the growth of spores of *B stearothermophilus* is a medium with the following composition: (in grams/liter of distilled water): K_2HPO_4 -- 30; KH_2PO_4 -- 10; NH_4Cl -- 5; Na_2SO_4 -- 1; $MgSO_4 \cdot 7H_2O$ -- 0.1; $MnSO_4$ -- 0.01; $FeSO_4 \cdot 7H_2O$ -- 0.01; $CaCl_2$ -- 0.001; yeast extract (Difco) -- 0.1% (by volume); trehalose -- 0.01 M; L-arginine -- 0.01 M; cobalamine -- 2 γ/l, pH 7.3.

Conclusions

1. Spore growth is stimulated by preliminary heat treatment at 100° for 10 minutes.

2. The concentration of amino acids, especially in a medium of L-arginine equal to 0.01 M, is most favorable for the growth of spores.

3. An increase in the concentration of carbohydrates in the medium from 0.001 to 0.01 and 0.1 M leads to an increase in the percentage of growth, with trehalose in a concentration of 0.01 M being most effective for growth.

4. Cobalamine (and to a lesser extent) thiamine and inosite are necessary for the growth of spores of *Bacillus stearothermophilus*.

5. The selected medium is optimum for the growth of spores of *Bacillus stearothermophilus*.

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STERILIZATION OF SPACECRAFT

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ABSTRACT: The first scientific successes in history involving the study of outer space have made real the possibility of detecting life on other planets. In this connection, the threat of contaminating other planets with terrestrial forms of life has arisen, especially microorganisms, prior to the performance of biological studies on those planets. The article treats certain aspects of international law as they apply to the problem of sterilization of spacecraft. Problems are discussed which relate to the determination of the resistance of microorganisms to unfavorable conditions of space, and various methods of sterilizing spacecraft used in the USSR and USA are discussed. An analysis of the data available in the literature shows that the problem of sterilization of spacecraft is being solved successfully at the present time.

Thanks to the considerable successes which have been attained in the study of outer space, it is now possible for the first time in history for scientists to make actual attempts to detect life on other planets.

However, at the same time that there have been successes in space technology, a real threat has arisen of contamination of other planets by terrestrial life forms, especially microorganisms, prior to the performance of biological studies on those planets.

On the basis of the assumption that life can arise and develop wherever appropriate favorable conditions exist for it, Lederberg and Cowie (1958), Davis and Communtzis (1960), Sagan (1960), Imshenetskiy (1962), Anders (1961) have studied in considerable detail the problem of possible biological contamination of the heavenly bodies by terrestrial life forms.

This problem is closely related to the solution of the problem of the origin of life. According to the theory that different planets may be at different stages of organo-chemical evolution, many scientists have expressed /185
the fear that even the exhaust gases of spacecraft falling into the atmosphere of another planet could lead to undesirable autocatalytic phenomena and thereby provoke certain artificial deviations which would disturb the

study of the specific conditions of a given planet, thereby disturbing the natural course of organo-chemical evolution on that planet.

However, the majority of investigators feel that there is insufficient basis for such fears, since penetration of negligible traces of dead organic matter can scarcely have a significant effect on the course of organo-chemical evolution of an entire planet.

However, there is an extremely great and real danger of the transfer of living substance, for example microorganisms. The situation might be otherwise in this case.

The transplanted microorganisms either might die if the favorable conditions required for their existence were absent or could enter a state of complete anabiosis. When the conditions on the planet are favorable for the multiplication of terrestrial microorganisms, the latter could either completely smother the aboriginal forms in the course of their multiplication or could exist parallel with them. In this case, the answer to the problem of the similarity or difference between life on Earth and that on other planets would be extremely difficult.

In connection with the development of the problem of sterilization of spacecraft, attempts have been made to do away with the need for sterilization of spacecraft, on the assumption that the extremely hostile conditions in space (high vacuum, ultraviolet and penetrating radiation, low temperatures) would have a profound effect on microbes that existed in the structure of the spacecraft. In answering the question as to whether the necessary sterilization of a spacecraft could be achieved in the course of its travel through interplanetary space, we must keep in mind the extreme resistance of microorganisms to extremal conditions in space. Thus, the classical experiments of Becquerel (1951) showed that superlow temperatures on the order of -272° and the conditions of a vacuum did not lead to the complete death of the organisms he studied. According to the data of Portner et al. (1961) and of Imshenetskiy and Lysenko (1964, 1965), certain microorganisms and their spores survived even better than in the control experiments in a high vacuum of 10^{-10} mm Hg and a temperature of approximately -20°.

Obviously, ionizing radiation cannot serve as a method of killing microorganisms in space. The doses found there are incomparably small relative to those which are undergone by bacteria that multiply in the water of atomic reactors.

Of all the extremal factors of the cosmic environment, those that are the most lethal to microorganisms are the ultraviolet rays. Doses of these rays that exist in outer space are absolutely lethal for all microbes. However, this effect is easily overcome by the slightest shielding. As indicated by the experiments by Fedorova (1969), a film of chromium only 800 Å thick provides reliable protection for spores of *Bacillus mesentericus* against the effect of UV-rays in a dose of $7.8 \cdot 10^7$ ergs/cm^2, equal to a lethal dose in space. /186

By means of direct exposure of microorganisms under deep space conditions, Hotchin et al. (1965) were able to show that various factors in the cosmic medium (high vacuum, cosmic and UV-radiation, X-rays and sharp temperature variations), all acting together, did not kill the test microorganisms.

Hence, the experimental data which we have regarding the survival of microorganisms under extremely unfavorable conditions in space indicate quite reliably that it will be necessary to perform careful sterilization of spacecraft that are intended to travel to other planets. Spacecraft require a constant temperature close to room temperature as well as normal pressure from precise operation of the apparatus, as well as sufficient protection against cosmic rays that prevents sterilization.

As far as the methods of sterilization are concerned, they were devised long ago and are widely used in biology, medicine and in certain branches of industry.

Consequently, the problem of sterilization of spacecraft involves a number of independent questions.

1. Theoretical reasons for the need to sterilize spacecraft. The effect of extremal factors of the space medium on microorganisms.

2. Methods and technology of sterilization of automatic spacecraft intended to travel to planets and land there.

3. Sterilization of manned spacecraft.

4. Sterile collection of soil samples from planets and the development of methods of studying them under sterile conditions.

5. Enforcing a quarantine on spacecraft and their crews following return to Earth from other planets.

Principles of Sterilization of Spacecraft

In the opinion of specialists who deal with the problems of sterilization of spacecraft (Jaffe, 1963a, b; Bruch, 1964; Sagan, Coleman, 1965; Magistrale, 1966; Astafyeva et al., 1967; Light et al., 1967; Horowitz et al., 1967; /187
Vashkov, Prishchep, 1967; Hall, 1968; et al.), the degree of sterility of a spacecraft is necessarily linked to the probability of contamination of the planet. In other words, a given spacecraft may be characterized as sterile only in the sense that the probability of infection from viable microorganisms from this spacecraft is quite low.

In 1959, Davies and Communtzis (cited in Horowitz et al., 1967) proposed a probability estimate of 10^{-6} as the acceptable degree of contamination of a planet by the landing of spacecraft. Somewhat later, Jaffe (1963 a,b), on the basis of calculations he has performed, reached the conclusion that the probability of contamination of the planet by automatic spacecraft sent to Mars according to his data must not exceed 10^{-4}. In his opinion, the possibility of contamination of Mars by successive manned spacecraft (an attempt at which will apparently be made between 1985 and 2000) is very great.

A detailed mathematical model of the quarantine policy from Mars was prepared later by Sagan and Coleman (1965). This work formed the theoretical basis of the COSPAR resolution on the necessity of sterilizing spacecraft, which was accepted in 1964 in Florence.

However, in the opinion of specialists, all of the parameters which were considered in the decisions of COSPAR, applied to an objective evaluation

and reevaluation. As new information is accumulated (Horowitz et al., 1967), changes must be made; at the special London Symposium of COSPAR (1967) on the methods of sterilization of spacecraft, appropriate corrections were made in the decisions adopted earlier by COSPAR.

According to the data of Hall (1968) and Imshenetskiy (1968), the mathematical equations for the probability of contamination of planets by microorganisms arriving together with spacecraft may be represented as follows:

$$P = n_1 P(n_i + n_u P(n_u) \tag{1}$$

or

$$P = P(n_m). \tag{2}$$

The probability of contamination of a planet by a sterilized apparatus is

$$P(n_1) = P(h);\ P(r);\ P(g) \tag{3}$$

whence

$$P(h) = P(N \geqslant 1) \tag{4}$$

If we consider that the possibility of landing is equal to unity, the probability of contamination of a planet by an unsterilized apparatus is as follows:

$$P(n_u) = \sum_{i=k}^{i=h} p(h')i \cdot p(r')i \cdot p(g')i, \tag{5}$$

where T - is the duration of the period of biological studies of the planet without human participation, during which it is necessary to prevent contamination; /188

P - is the probability of contamination of the planet during time T;

n - number of spacecraft which land or fall on the planet during time T;

$P(n_i)$ - average probability that one out of n_1 craft which land could cause contamination of the planets;

n_u - number of craft which do not make landings (orbital flights, fly-by mission);

$P(n_u)$ - mean probability that one of the craft which does not land will cause contamination of the planet;

n_m - number of spacecraft sent to the planet, either to land or not;

$P(n_m)$ - mean probability that sending n_m craft will cause contamination of the planet;

N - number of living microorganisms present;

N_0 - initial number of cells of living microbes prior to sterilization;

$P(N \gg 1)$ or $P(N = 1)$ - probability that one or more microbes will be present;

h - transfer of living microorganisms to a planet, leading to its contamination;

ph - probability that h will take place;

r - landing on the planet or entrance into its atmosphere of organisms which survive sterilization;

p(r) - probability that r will take place;

r' - penetration to the surface of the planet or into its atmosphere of an organism or organisms which have not been subjected to sterilization;

p(r') - probability that r' will take place;

g - growth and spread over the surface of the planet or in its atmosphere of terrestrial microorganisms which survive sterilization;

p(g) - probability that g will occur;

g' - growth and spread over the surface of the planet or in its atmosphere of terrestrial microorganisms which have not been subjected to sterilization;

p(g') - probability that g' will take place.

An appropriate analysis of all the conditions and experimental data on the content of microorganisms in spacecraft and the effectiveness of various methods of sterilization has made it possible to conclude that the probability of contamination of the planet by terrestrial microorganisms will not exceed 10^{-3}

In order to satisfy this requirement, the spacecraft capsule must be made of materials which contain a minimum quantity of microorganisms, and under /189

conditions which reduce the content of microorganisms and therefore can be subjected to complete sterilization. In addition, the capsule intended for sterilization must be microbiologically isolated from any unsterile portions of the spacecraft prior to the moment of separation from the last stage of the booster rocket during the approach to the planet.

Morbidity of Microorganisms During Sterilization

Although the mechanisms of the death of microorganisms differ for various methods of sterilization, the actual course of the process, in the opinion of many specialists, is frequently quite uniform, regardless of what agent is used to achieve sterilization. The process of the death of microorganisms frequently follows an exponential law (i.e., at a constant rate in equal time intervals). This occurs so frequently that some investigators have concluded that there must be some common process which lies at the bottom of the action of all sterilizing agents.

On this basis, Magistrale (1966) suggests that it would be possible to establish a direct relationship between the rate of death of microbes and the time of action of thermal, chemical or radiation sterilization. Equal fractions of the microbial population will die in equal time intervals.

According to Magistrale's calculations, the decimal time of reduction of the number of living microbes in the sterilization process is determined by the magnitude of the time interval during which 90% of the microflora die under given sterilization conditions. The parameter, called the D parameter, is considered as the time required to kill any specific microorganism with a probability of 0.9.

According to the data of Magistrale, the parameter D may be calculated for certain bacterial spores for the sterilization process using dry vapor as solvents:

Time, Minutes	Number of Organisms Killed	Number of Organisms Which Survived
0	0	1,000,000
1	900,000	100,000
2	990,000	10,000
3	999,000	1,000
4	999,900	100
5	999,990	10
6	999,999	1

These data show that for a concrete sterilized sample containing 10^6 /190
microorganisms and a parameter D equal to one minute, 999,999 organisms will die by the end of the sixth minute and only one organism will theoretically remain alive.

What is the probability that this organism will survive longer than six minutes? After heating for four minutes, 999,999.9999 organisms will theoretically be dead and 0.0001 will be alive. Obviously, this satisfies the original requirement established for the probability of contamination. Since the duration of sterilization depends on the initial number of microorganisms, it is necessary to know the number of microbes on the external and internal surfaces of the apparatus. The number of microbes on the surface depends on the conditions under which preparation of the apparatus takes place, as well as on the contamination of the materials and the devices that go into its construction.

To calculate the total number of microorganisms prior to sterilization, it is necessary to determine the numbers on the surface of the materials, devices and assemblies of a spacecraft.

Detection of Microorganisms in Various Materials

In the standard materials which are usually encountered in design, the contaminating bacterial are found fairly easily. However, rapid development of space technology, intended for the investigation of planets, is placing new requirements on microbiologists. Determination of the presence of microbes in such materials as plastics, epoxy resins, fuel, etc., is very important for evaluating the results of tests following sterilization. The surfaces of these materials may be studied in various ways, but the content

of microbes inside the materials is extremely difficult to determine. It is necessary to work out special methods of investigation.

A great deal of work has been done in this area in the United States, where methods are used to detect living organisms in solid materials. Opfell and Bandaruk (1966), in order to obtain a crushed sample, compared such methods as drilling, crushing in a ball mill, crushing in a mortar, as well as solution of solid materials. The most effective method proved to be drilling and the study of the particles thus formed for the presence of bacteria.

The principal goal of all of these experiments had to do with the detection of microorganisms (regardless of their physiological properties), found inside various materials that were used in the assembly of spacecraft.

Similar work has been performed in the Soviet Union. In order to detect microbes inside materials under study, under conditions which completely exclude the penetration of other microflora into the inner layers of a sample, /191 the Institute of Microbiology of the Academy of Sciences of the USSR devised a method and built a box with special drilling apparatus. A detailed description of the box is given in the work by Imshenetskiy and Abyzov (1966).

Special methods of sterilization were used for the surfaces of the investigated samples prior to inserting them in the chamber and special methods for sterile collection of inoculation material from the internal layers of the investigated samples. The box makes it possible to carry out microbiological analysis of thermoresistant materials only. Figure 1 shows the process of roasting the sample prior to insertion into the chamber.

In order to avoid transfer of microbes during drilling from the surface /193 layers to the interior, the sample is initially split in the first chamber into two halves. Then the two halves are transferred to a second chamber with the surface of the split placed against the flame of a burner. The opening of the hatch is then screwed down. Drilling is performed on the surface of the split, in the central portions of it (Figure 2).

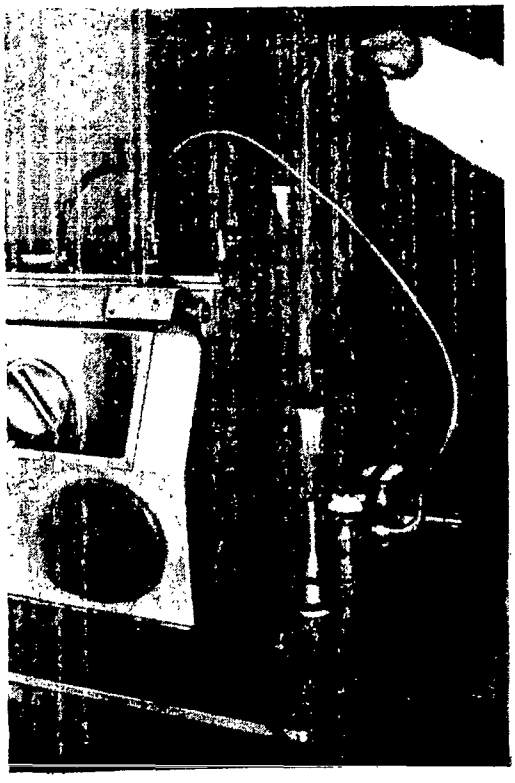

Figure 1. Firing of the Sample Prior to Insertion Into the Chamber.

The drilled-out material from the sample under study obtained in this fashion is used as the inoculation material, which is transferred on the tip of a scalpel into the nutrient medium (Figure 3).

In a qualitative estimate of the microorganisms contained within various samples, special scales for weighing batches of inoculation material are placed inside the box.

The studies of Astafyeva et al. (1967) have shown that the content of microbes within the material or their absence is determined both by the technology of the production of the material itself as well as by its density and porosity, on which the degree of penetration of microbes into the depths of the material depends. These authors state that "although the technique of preparing many materials ensures their complete sterility, subsequent handling of such materials and the manufacture of parts (joining of surfaces, details, assemblies) can lead to hermeticization of microflora and a transfer of the culture from the surface to the interior, if corresponding measures for sterilization are not taken."

These authors performed an inoculation with samples collected from the inner layers of 86 radio-electronic elements on various nutrient media. However, no microorganisms were found. The authors, however, do not rule out

the possibility of the existence of viable microorganisms within certain of the radio-electronic elements which they studied.

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Figure 2. Samples to be Investigated Following Removal of Samples from the Central Portions.

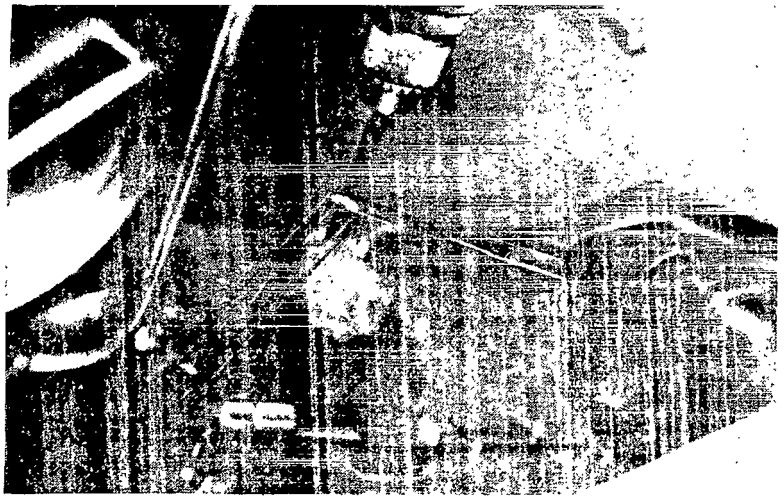

Figure 3. Inoculation of a Sample In The Sterile Chamber

In this connection, we should point out the importance of the sensitivity of the method of detecting microorganisms. Many investigators have pointed out that the sensitivity of the method of detecting the viable microorganisms depends on the type of the material being studied. For example, Opfell and Bandaruk (1966) feel that negative results are still not a reliable indication of the sterility of the investigated material. In their opinion, in the absence of a highly sensitive method of detecting microbes in certain cases, a conclusion regarding sterility may be reached on the basis of indirect data, for example on the basis of information regarding the origin of this material or the technology of its preparation. On the basis of these considerations, we can assume that metals of different types and their alloys, certain industrial rubbers, textolites, fire-resistant materials and certain synthetic materials are sterile inside.

By selecting appropriate materials with a consideration of the degree of /194
their contamination by microorganisms, it is obvious that we can reduce to a minimum the content of microorganisms inside the materials that are used for building spacecraft.

Many investigators (Davies, Communtzis, 1960; Davies, Horowitz, 1966; Astafyeva et al., 1967; Vashkov, Prishchep, 1967; etc.) feel that the process of preparations and assembly is the most important source of contamination of a spacecraft by microorganisms.

Hence, even the most superficial analysis of the data on microbial contamination of various materials, structures, and assemblies as well as the spacecraft as a whole indicate that in order to achieve complete sterility it is necessary to use a number of appropriate methods of sterilization that are used at various stages of technology in the preparation of a spacecraft.

Thermal Sterilization

At the present time, there is no uniform method of sterilization of a spacecraft by means of dry heat.

American scientists feel that pharmaceutical devices and materials must be sterilized with dry heat for two hours at 170°. In England, it is felt

that such sterilization of vessels, ampoules, etc. must be performed in one hour at 150°. It is official policy in the USSR that heating in a chamber for one hour at 160-170° is sufficient to achieve reliable sterilization.

The differences that have been noticed in the times and temperatures can be explained partly by practical experience and the method of performing sterilization by dry heat in these countries, the use of different test microorganisms, and the degree of contamination of the apparatus to be sterilized (Bruch, 1964).

The existence of a large amount of non-heat resistant materials in spacecraft does not allow the use of generally accepted methods of thermal sterilization. In the available literature, until recently there were no data on the sterilization process using dry heat at low temperatures on the order of 110-140°. Therefore, both we and those working abroad in recent years have intensified our efforts to find the most effective methods of sterilization using dry heat at lower temperatures (110-140°).

Studies in this regard were begun with the isolation from nature of bacteria with the most heat-resistant spores.

According to the data of Bruch (1964), after heating two soil samples at a temperature of 120 to 160°, spore growth was observed and those cultures were isolated which were grown later on under laboratory conditions.

These studies showed that the formerly recommended method of heat /195
sterilization of spacecraft at 125-130° was insufficiently effective. By using various sterilization methods, cultures were isolated that possessed increased resistance to high temperature in comparison to the other sporogenous forms.

Another worker in the same laboratory, Koesterer (1964), later succeeded in isolating from soil still more heat resistant forms of mesophyllic aerobic sporogenous bacteria.

Heat-resistant spores of microorganisms were then placed on various materials (paper strips, asbestos coatings, plaster, adhesive dry stucco), and these infected materials were subjected to sterilization by dry heat at 120°.

The experiments showed that various solid materials increased the resistance of spores of *Bacillus subtilis* var. *niger* to sterilization by dry heat. The same effect was obtained by these authors when testing certain other strains of sporogoneous bacteria.

Astafyeva et al. (1967) obtained a heat-resistant spore test culture isolated from soil, on metal disks and certain materials used in the construction of spacecraft, and subjected these tests to the action of temperature in the range from 100 to 200°.

In their experiments, the death of test cultures on metal plates at 100-102° took place in 12 hours, in 11 hours at 130°, and in 15 minutes at 180°. The authors feel that it is necessary to take into account the time required for heating the disks to the appropriate temperatures. The duration of sterilization of certain parts depends on the nature of the material, their thermal capacity and thermal conductivity, as well as the weight and the shape of the part.

Experiments were performed at the Institute of Microbiology of the Academy of Sciences of the USSR to clarify the method to be used for sterilization of infected materials which differ sharply in their characteristics at temperatures of 102-105°.

Spores of *Bacillus mesentericus* and *B. subtilis* were transported to tin disks, pieces of calico and poroplast.* The results of the experiments are shown in the table.

As we can see, the material that was most difficult to sterilize is poroplast, and consequently, in order to establish the period of exposure, it is necessary to proceed on the basis of the existence of the difficult sterilized materials which are found in the spacecraft.

The results in this table also indicate that the resistance of the spores to high temperatures may be also affected by various properties of the test carriers.

*Translator's Note: translation unknown; literally "a porous layer."

In the opinion of Bruch and Koesterer (1962), soil and certain other test carriers (under equal conditions of humidity) make sporogenous forms more resistant to the action of dry heat.

STERILIZATION WITH DRY HEAT AT A TEMPERATURE OF 102° OF MATERIALS CONTAINING SPORES OF *BACILLUS SUBTILIS* AND *B. MESENTERICUS* /196

Material studied	Exposure time, days											
	7	8	10	15	17	20	7	8	10	15	17	20
	Experiment						Control					
	Bacillus subtilis											
Poroplast	+	+	-	-	-	-	+	+	+	+	+	+
Cloth (calico)	+	-	-	-	-	-	+	+	+	+	+	+
Metal (iron)	+	+	+	-	-	-	+	+	+	+	+	+
	Bacillus mesentericus											
Poroplast	+	+	+	+	+	-	+	+	+	+	+	+
Cloth (calico)	+	-	-	-	-	-	+	+	+	+	+	+
Metal (iron)	+	+	+	+	-	-	+	+	+	+	+	+

Hence, the results of the tests which were performed indicate that by using heat-resistant materials and also reducing the temperature for sterilization in order to lengthen the period of exposure, we can create effective methods of thermal sterilization for the spacecraft as a whole.

Bruch (1964) describes the sterilization of American spacecraft using dry heat at a temperature of 135° for 22 hours.

According to the data of Lorsch and Koesterer (1967), with the method currently used in the USA for sterilizing with dry heat, spacecraft are subjected to 160° for three hours or to 105° for two weeks.

Radiation Sterilization, Filtration and the Use of Bactericidal Autosterilizers

As we know, two methods are suitable for sterilization of apparatus and materials: 1) heating to 130-180° and 2) irradiation with gamma-rays. However, in many cases heating to these temperatures causes damage to electronic apparatus. Therefore, it becomes necessary to use sterilization with gamma-rays.

The method of sterilization using gamma-rays is enjoying increasing popularity nowadays in medical practice and the food industry. A combined effect of dry heat and ionizing radiation is very promising as far as sterilization of certain hermetically sealed radio parts is concerned.

Bruch (1964) and Koesterer (1964) showed that a positive effect may be obtained either by alternating the action of dry heat and gamma-radiation or by simultaneous action of these factors. /197

According to the data of Jaffe (1963 a, b), the dose required for sterilization of materials and apparatus on a spacecraft is 10 million rads. However, Astafyeva et al. (1967), feel that these doses are much too high. According to their data, sterilization of radio parts containing spores of *B. mesentericus* which are resistant to the action of gamma-rays can be accomplished with a dose of 2.5 million rads. The same dose proved to be effective for sterilization of porous rubber and certain liquids that were considerably contaminated by a test culture. Special tests of sterile radio parts and materials for various storage times (from one day to three months) showed that the radio-technical parameters of the devices and the physical and chemical properties of the materials remain unchanged. The studies that were performed indicated that the use of radiation sterilization in many cases is necessary and completely permissible in the process of sterilization of spacecraft.

With production of certain sterile systems of spacecraft and the maintenance of sterility of these systems require sterilization by means of filtration.

Filtration is widely used for sterilizing liquids and gases. However, in the opinion of the investigators who deal with problems of sterilization of spacecraft, the use of membrane filters is not advantageous, since they are frequently damaged in the course of filtration. Astafyeva et al. (1967) used asbestos filters for sterilizing liquids; these filters are also used in medical practice. She confirmed that a single filtration of a liquid containing one billion cells per ml requires effective sterilization of the liquid; the same effect was obtained with liquids exhibiting various degrees of viscosity. Viscosity affects only the duration of the filtration process.

As far as sterilization of gases is concerned, the passage of various gases and their mixtures through special sterile filters which hold back microparticles ensures completely sterility of these gases (Jaffe, 1963 a, b; Lev, 1964).

In the process of sterilization of spacecraft, specialists have frequently encountered difficulties due to the fact that certain materials caanot be sterilized by the necessary doses of radiation, nor by temperatures that would ensure the necessary degree of sterility. Therefore, specialists who work with problems of sterilization of spacecraft are particularly interested in a study of the bactericidal, autosterilizing that are used on spacecraft.

Special studies by Astafyeva et al. (1967) have shown that various materials that are used for making spacecraft (alloys of Mg, Al, Cu, Ag, surfaces covered with silver or copper, certain types of rubber) as well as oxide films that are produced under industrial conditions possess antimicrobial activity. When using certain enamels, the microbial infection is reduced by tens of thousands of times. However, the test culture remains viable beneath the coatings of paint and adhesive.

According to the data of Shank et al. (1962), Zsolnai (1962), Godding and Lynch (1965), the liquid fuel which is used in spacecraft possesses high bactericidal, autosterilizing properties to varying degress, while the solid fuel components do not possess such properties.

In this connection, particular emphasis was placed on studies in which certain materials and compounds used in space technology were given bactericidal and autosterilizing properties. Opfell (1963) presents data indicating that when small amounts of paraformaldehyde were added to dyes, plastics, greases, and other compounds, they acquired bactericidal and autosterilizing properties. Willard and Alexander (1964) showed that the addition of 3-7% formaldehyde to various dyes and coatings confers bactericidal properties on these materials. Vashkov and Shcheglova (1968), in order to obtain bactericidal cellulose cloth used on a spacecraft, combined the macromolecule of cellulose chemically with various bactericidal metals, quaternary-ammonium bases and phenol preparations. These materials produced the death of 70-100% of gram--positive and gram-negative microorganisms that were placed on their surfaces.

Hence, it was shown experimentally that by using various methods it is possible to achieve sterilization of any material or device used on a spacecraft.

Sterile Assembly

With satisfaction of all the requirements of sterility which apply to devices, materials, connectors, joints, lubricants, coatings, etc., the principal source of microbial contamination of a spacecraft is the hands of the engineering and technical personnel under conditions under which the final assembly of the object as a whole is performed. Therefore, in the unanimous opinion of the specialists who work on the problem of sterilization of spacecraft, assembly must be carried out with observation of the same precautions that are usually used in a surgical or bacteriological operation. /199 However, it must be emphasized that the absence of pathogenic microflora in the operating area does not mean total sterility of the area. In other words, the requirements for sterility of the area, instruments, hands and clothing of the personnel when assembling spacecraft must be stricter. Jaffe (1963 a, b) feels that when liquid bactericidal agents are used to clean surfaces during sterile assembly, the possibility of bacterial contamination is from 10^{-1} to 10^{-2} particles. However, if the treatment with bactericidal fluids is accompanied by a subsequent radiation with ultraviolet light, there is a further sharp decline in the possibility of contamination of devices, instruments and materials by microbes.

In the studies of Vashkov et al. (1968), hydrogen peroxide was used to sterilize the surfaces of the materials, devices and working areas. It was shown that this substance is effective with respect to vegetative cells in a concentration of 3-5%, 3-10% for spore forms and 1-5% for viruses. The shortcomings of hydrogen peroxide listed by the authors include a high surface tension of 73 dynes/cm. However, when mixed with detergents, this property of hydrogen peroxide disappears.

The purity of the air in the area is ensured by filtering the air which is supplied to the area through special filters.

Sterile assembly of individual assemblies and parts is carried out by using special sterile boxes fitted with rubber gloves. The principles of the construction of various boxes for such purposes are described in papers by Webb and Softki (1953), Phillips et al. (1955), Trexler and Reinolds (1957), Imshenetskiy and Abyzov (1966) and Phillips (1966).

A detailed description of the methods of determining and considerably reducing the number of microbes in areas for the sterile assembly of spacecraft as well as the construction of special boxes and areas for these purposes is given in a work by McDade et al. (1968).

However, it should also be pointed out that following sterilization of all parts of a spacecraft and sterile assembly, we still cannot exclude the possibility of microbial contamination of open surfaces and sections of some parts. Therefore, for a final sterilization, it is necessary to use a method which will provide maximum bactericidal properties but will not have a harmful effect on any of the structures or materials of the finished product. Hence, gas sterilization is employed.

Gas Sterilization /200

In addition to the thermal and radiation types of sterilization, as well as filtration of gases and liquids, and the use of bactericidal, autosterilizing materials and sterile assembly during sterilization of spacecraft, the gas method can be used. The high level of effectiveness of this method as far as sterilization of individual materials, assemblies that would be destroyed if other methods of sterilization were used is concerned, as well as in the final sterilization of a spacecraft as a whole following its assembly, the gas method provides one of the basic methods of sterilization of spacecraft at all stages of its manufacture.

Phillips and Hoffman (1960), Davies and Communtzis (1960) suggested the use of ethylene oxide for gas sterilization of spacecraft; this substance is very effective because of its high bactericidal properties and considerable penetrating ability.

Methods of using ethylene oxide for disinfection and sterilization in various areas, the mechanism of its bactericidal action on microorganisms, etc., have been studied in detail in survey articles by Phillips and Kaye (1949), Bruch (1961) and Kelsey (1967).

A mixture of ethylene oxide (3 to 90%) with air is highly explosive. Hence, ethylene oxide is usually used in a mixture with such explosion-proof gases as carbon dioxide, freon and other compounds. These mixtures penetrate readily into many materials -- paper, cloth, certain plastics, rubber.

However, many authors point out that the method of gas sterilization does not allow effective sterilization of the entire thickness of many plastics and the cavities of closed volumes. In this connection, while working out methods of gas sterilization, considerable emphasis has been placed on a study of the processes of diffusion of bactericidal gases into closed systems of spacecraft. In recent years, while developing methods of gas sterilization, several authors including Opfell et al. (1964), Vashkov and Prishchep (1967) have carried out special investigations.

According to the data of Opfell (1963), Jaffe (1963 a, b), Davies and Communtzis (1960), a gas mixture is used for sterilization of spacecraft in the USA which consists of 12% ethylene oxide and 88% freon 12 (so-called cryoxide).

Vashkov and Prishchep (1967) have proposed the use of a new more effective mixture for the sterilization of spacecraft. This mixture which consists of 40% ethylene oxide and 60% methyl bromide has been called "OB" mixture by the authors. According to the data of these investigators, a mixture of this kind exceeds cryoxide in terms of its bactericidal properties due to the high bactericidal capacity of both of the components. Thus, the "OB" mixture is /201
five times more effective in its action on *Staphylococcus aureus* and 2.5 times more effective against spores of *B. mesentericus* than a mixture containing 12% ethylene oxide and 88% freon. In the course of special studies, these authors demonstrated that the "OB" mixture will not harm materials being sterilized.

The complete removal of the sterilizing mixture following sterilization is very important in gas sterilization of spacecraft. Complete removal of the bactericidal mixture is important both for the collection of samples during the microbiological check of sterility and to ensure normal operation of individual devices during flight and subsequent experiments on the surface of the planet to be studied. In this connection, special emphasis is placed on problems of diffusion, adsorption and desorption of the sterilizing gases, and these problems are being solved successfully at the present time.

Technical problems that are related to the manufacture of sterile spacecraft intended for studying other planets may be solved successfully in practice if the necessary technological conditions are maintained at all stages of assembly of the apparatus and in the place where the instruments are located. It should be emphasized that the technology of sterilization of spacecraft, proceeding at a very rapid rate these days, will be improved and simplified in the future.

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